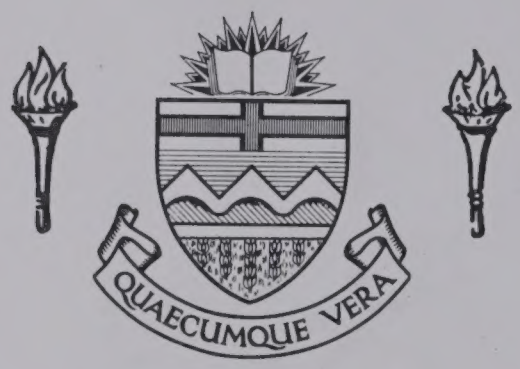

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THE UNIVERSITY OF ALBERTA

THE SPECTRAL SENSITIVITY OF *HYDRA CARNEA* L. AGASSIZ (1850)

by

VIRGINIA LEE ELLIS

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR

THE DEGREE OF MASTER OF SCIENCE

DEPARTMENT OF ZOOLOGY

EDMONTON, ALBERTA

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies for acceptance, a thesis entitled "The Spectral Sensitivity of Hydra carnea L. Agassiz (1850)" submitted by Virginia Lee Ellis in partial fulfilment of the requirements for the degree of Master of Science.

ABSTRACT

The spectral sensitivity of *Hydra carnea* was investigated by means of growth rate and action spectrum studies. The rate of asexual growth was measured over a 40 day period for animals cultured under white, blue, green and red lights of equal intensity. The growth rate was greatest under red light and slowest under blue; the rate under white light was similar to that under green. The rate of growth was found to be proportional to the number of quanta available under each light.

The action spectrum, measured as the reaction times to light stimuli of various wavelengths, varied according to the light under which the animals were cultured. For animals that had been cultured in the dark, the reaction time was shortest at 650 nm and longest at 450 nm. Animals cultured under blue, green and red lights showed a decreased sensitivity, indicated by an increased reaction time, to the light under which they were cultured as compared to animals cultured in the dark.

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I would like to thank Dr. D. D. Beatty and Dr. J. K. Lauber for helpful discussions during the course of this research. I also thank Dr. D. D. Beatty, Dr. G. O. Mackie and Dr. P. E. Hallett for their critical reviews of the thesis.

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I. INTRODUCTION

General Nature of Hydra

Hydra is a genus of freshwater cnidarians with 15 species known to exist in the ponds and streams of North America (Forrest, 1963). Credit for the discovery of hydra* goes to Antony van Leeuwenhoek (1702), but it was not until Abraham Trembley's work (1744) that any extensive observations were made on the animal. Trembley performed regeneration and grafting experiments on hydra and studied its asexual reproduction and its phototaxis. His observations and experiments are considered classics in the field. Surprisingly, it has only been within the last twenty years that hydra has been used extensively for biological research, as was presented in Lenhoff and Loomis (1961).

The basic biology of hydra is treated in nearly all invertebrate textbooks, with particularly good accounts given in Meglitsch (1967) and Barnes (1963), so it will not be discussed here. Hydra has several characteristics which make it an excellent animal for the investigation of

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*In this text, hydra is used as the common name for specimens of the genus *Hydra*. Following the terminology of Lenhoff and Loomis (1961), hydra is used in the singular to indicate one or more specimens of a single species or of *Hydra* in general when species have not been indicated.

biological phenomena. Being a small organism, it can be cultured in the large numbers necessary for quantitative studies or for biochemical analysis such as the study of the carotenoids of hydra by Krinsky and Lenhoff (1965). The presence of continuous cell division in hydra facilitates studies of growth, regeneration and cellular differentiation, such as the investigations of Burnett (1961), Diehl and Burnett (1965b), and Campbell (1967). Asexual reproduction by budding is a process which under favorable conditions takes only two days for completion in hydra (Hyman, 1940). This process has been studied by Diehl and Burnett (1965a) and by Clarkson and Wolpert (1967), and it permits one to obtain clones of genetically identical individuals. The simplicity of its structure, consisting of external and internal epithelia separated by a mesoglea, means that hydra lacks "a definite self-regulated internal extra-cellular fluid" (Lenhoff, 1961); both cell layers are bathed by the environmental fluid. Thus, one can exactly control the environment of the cells for studies of phenomena such as respiration (Lenhoff and Loomis, 1957), osmoregulation and ionic regulation (Lilly, 1955), or the feeding response of hydra (Lenhoff, 1965).

Photophysiology of Hydra

The earliest observations on hydra in regard to light were made by Abraham Trembley (1744). He found that the

animals accumulated on the illuminated side of their container. The next significant work on this positive phototaxis was by Wilson (1891), who noted that Hydra viridis and H. fusca were most sensitive to blue light. He placed colored glass plates on the illuminated side of an aquarium and found that most of the hydras accumulated under the blue light. In 1960, Feldman and Lenhoff investigated the phototaxis of H. littoralis. They found that recently fed hydra showed no phototactic response. Animals starved two days gave a variable response, but starvation for four or more days gave an increasingly positive response. After seven days without food, the animals moved toward the light at the rate of one centimeter per hour.

Feldman and Lenhoff also noted that each hydra oriented its hypostome and tentacles toward the light. If an animal was sectioned below the hypostome, the upper end moved toward the light while the lower end did not. These observations suggested localized photoreception at the apical end rather than a generalized photosensitivity. Animals that had been starved for seven days were depigmented except for a deep orange area at the hypostome. Alcohol extracts of this pigment had an absorption spectrum similar to astaxanthin, a carotenoid, but no evidence could be found to indicate that it served as a photoreceptive pigment in hydra (Feldman and Lenhoff, 1960).

Haug (1933), studying H. viridis and H. oligactis, was

the first to note that the animals reacted to a light stimulus by first contracting and then orienting toward the light. Rushforth, Burnett and Maynard (1963), studying the contraction response of H. pirardi to light stimuli, found that the contraction time was inversely related to the intensity of the light and that there was no habituation to a light stimulus. The response time was also wavelength specific with the minimum contraction time in response to blue light. In addition, the light response could be inhibited by removal of the hypostome and tentacles. Rushforth, Krogh and Brown (1964) found that the light response of H. pirardi could also be inhibited by feeding the animals live Artemia salina, or by adding a homogenate of A. salina or a 10^{-5} M solution of reduced glutathione to the hydra culture medium. After feeding, the contraction response was gradually restored over a period of 50-60 minutes.

Singer, Rushforth and Burnett (1963) studied the contraction response of Hydra pirardi to light stimuli of various wavelengths. Measuring the time between onset of the light stimulus and contraction of the animal, they found that the response time was shortest in blue light and that there was a sharp decrease in sensitivity above 500 nm.

Singer (1964) found that the contraction response of H. pirardi was inhibited by atropine, an inhibitor of acetylcholine, and was enhanced by methacholine chloride, which is similar in structure to acetylcholine.

Physostigmine and neostigmine, both inhibitors of cholinesterase, also enhanced the contraction response. These results indicated that the contraction response was mediated by the nervous system through a mechanism involving acetylcholine.

Electrophysiological studies have indicated that hydra have two major coordinating systems, each with pacemakers (Passano, 1962). The first system showed repetitive compound pulses called rhythmic potentials, which were up to 0.2 mV in amplitude. These potentials have not been directly correlated with any observable behavior of the animals. The second system, called the contraction burst system, appeared to control the periodic coordinated contractions of the epidermal longitudinal muscles by means of slow compound potentials, with amplitudes of 0.3-0.6 mV, preceding the contractions.

Passano and McCullough (1962) made electrophysiological studies of the light response of H. pirardi. They found that a light stimulus temporarily interrupted the rhythmic potentials and then caused a shift to a new pacemaker site accompanied by an increased frequency of the rhythmic potentials. The light did not affect conduction in the coordinating system but seemed only to affect the pacemaker. Further study (Passano and McCullough, 1964) showed that a light stimulus also interrupted a contraction burst in progress and then caused an increased rate of contraction

bursts. The rate began to decline after about 20 minutes. They also found that contraction burst frequency showed a diurnal rhythm with the lowest rate prior to dawn, the highest after sunrise, and a gradual decline during the day. The daylight frequency was 1.5-2 times the frequency at night. Passano and McCullough (1965) also found that the rhythmic potential system was most sensitive to blue light, showed a sharp decline in sensitivity at 500 nm and appeared to be insensitive to red light.

This study was undertaken to learn more about the spectral sensitivity of hydra. Earlier studies showed that hydra were most sensitive to blue light but nothing was mentioned concerning the environmental light under which the animals were cultured. The purpose of this study was to yield additional information on the photophysiology of hydra by determining the relationship between spectral sensitivity and environmental light, as indicated by growth rate and action spectrum studies.

II. METHODS AND MATERIALS

Collection and Identification of Animals

The hydra used in this study were collected on September 9, 1967, from the outlet canal of the Calgary Power Plant at Lake Wabamun, Alberta. This site was chosen for its accessibility and abundance of fauna. The water, being warmed by the power plant, does not freeze in winter thus making collection possible at any time of the year. The lake is located at 53°32' North latitude, 114°35' West longitude, which is approximately 40 miles west of Edmonton on Highway 16. The altitude is 2378 feet.

The animals were found by examining littoral plants in the canal. The plant from which the hydra for this study were collected was pondweed, Potamogeton pectinatus L. The hydra densely covered the plant at a depth of 2-30 centimeters. At the time of collection, the water temperature was 22°C and the pH was 7.9. To collect the hydra, pondweed was placed in glass containers with canal water and transported directly to the laboratory. These vessels were aerated for several days, and the animals were transferred to culture dishes as soon as they were found detached from the plant material. Clones were established with a number of these hydra by placing one individual in each dish and allowing it to reproduce asexually by budding.

Of the six species of Hydra known to occur in Alberta, the species used for this study was Hydra carnea L. Agassiz (1850). Identification of the hydra was based on the size of the tentacular nematocysts, the character of the sexual state, and the shape of the embryonic theca. The lengths of the tentacular nematocyst capsules were measured using bright-field microscopy and an eyepiece micrometer. Twenty nematocysts of each type were measured to ensure an adequate sample for identifying an individual, and five animals were used to identify a clone. The measurements obtained were then compared to the size ranges for all the species of hydras in Alberta as determined by Adshead, Mackie and Paetkau (1963). Table I shows the size ranges for the nematocysts of H. carnea.

Table I

Nematocyst capsule lengths for H. carnea. Measurements are in microns (μ).

Nematocyst Type	Adshead et al. (1963)	Present Study
Desmonemes	6.0-9.0	6.3-7.9
Stenoteles	10.5-20.0	11.0-13.4
Atrichs	7.5-11.5	7.7-9.6
Holotrichs	9.0-12.5	9.4-11.4

The identity of this species was confirmed by comparison with the description by Hyman (1931). She characterized

the species by its relatively slender column about ten millimeters in length. Tentacles may be 1.5-3 times the column length. The animal is reddish brown. It readily becomes sexual in the laboratory and is hermaphroditic, exhibiting protandry. The nippled testes are located on the upper column while the ovaries develop more proximally (Figure 1). The embryonic theca, also shown in Figure 1, is spherical with short thick spines. The nematocysts of H. carnea are illustrated in Figure 2. The holotrichs of this species are especially characteristic with three distinct transverse coils of the thread located anteriorly.

Culture Methods

Special care must be taken to maintain large numbers of hydra in optimum condition in the laboratory. Hydra are extremely sensitive to environmental changes and may elapse into a state of depression, accompanied by degeneration and death, if conditions are not carefully controlled (Loomis, 1953). The animals cannot be cultured in tap water without use of a chelating agent due to the toxicity of copper ions (Chalkley and Park, 1947). Distilled water is insufficient for culture because hydra need both calcium ions (Loomis, 1954) and sodium ions (Lenhoff and Bovaird, 1960) in their environment. Conditions for optimum growth of hydra have been defined by Loomis (1954), and a satisfactory culture method has been established by Loomis and Lenhoff (1956),

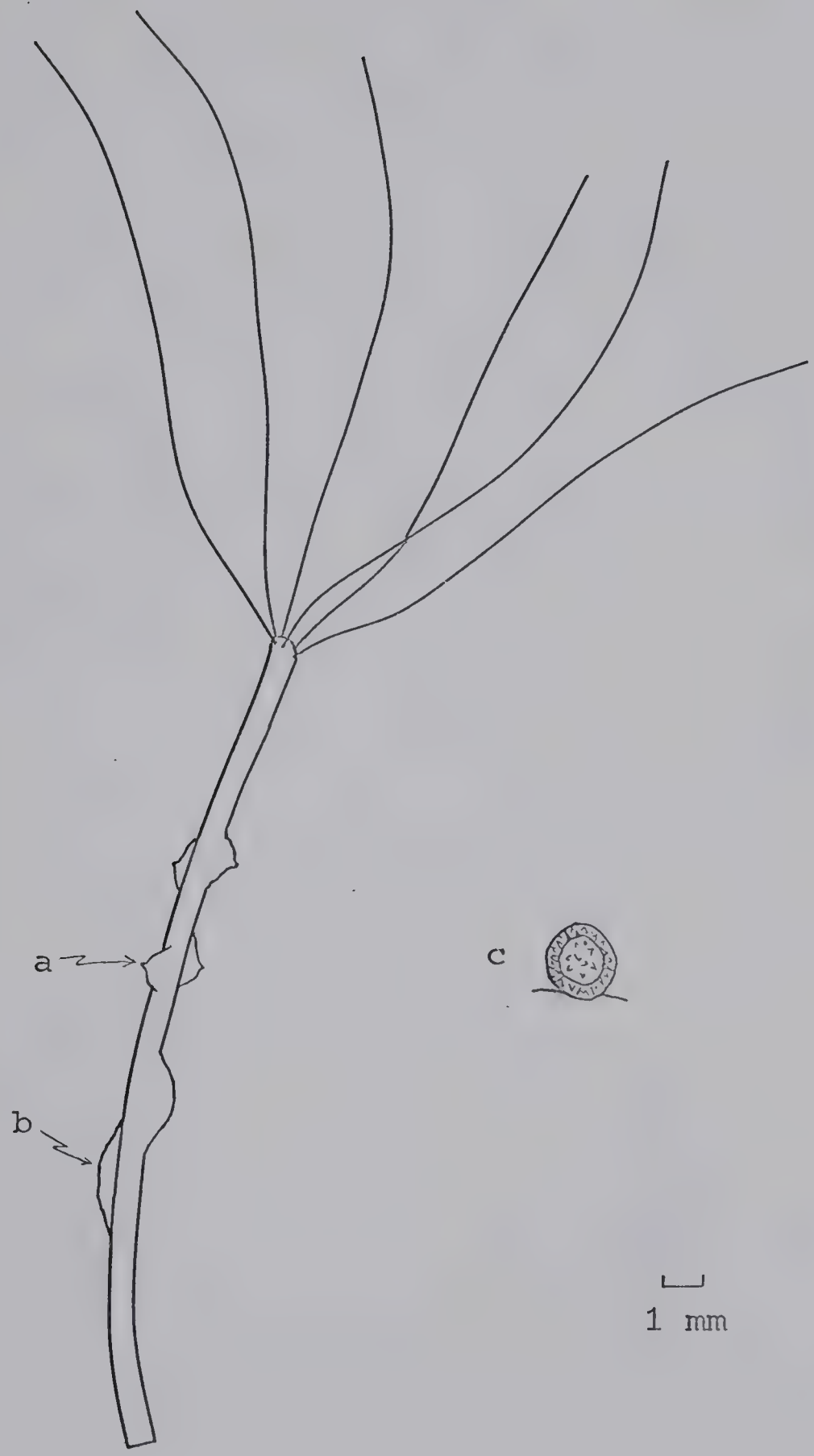

Figure 1. Drawing of Hydra carnea in the sexual state, showing (a) testis; (b) immature ovary; (c) embryonic theca.

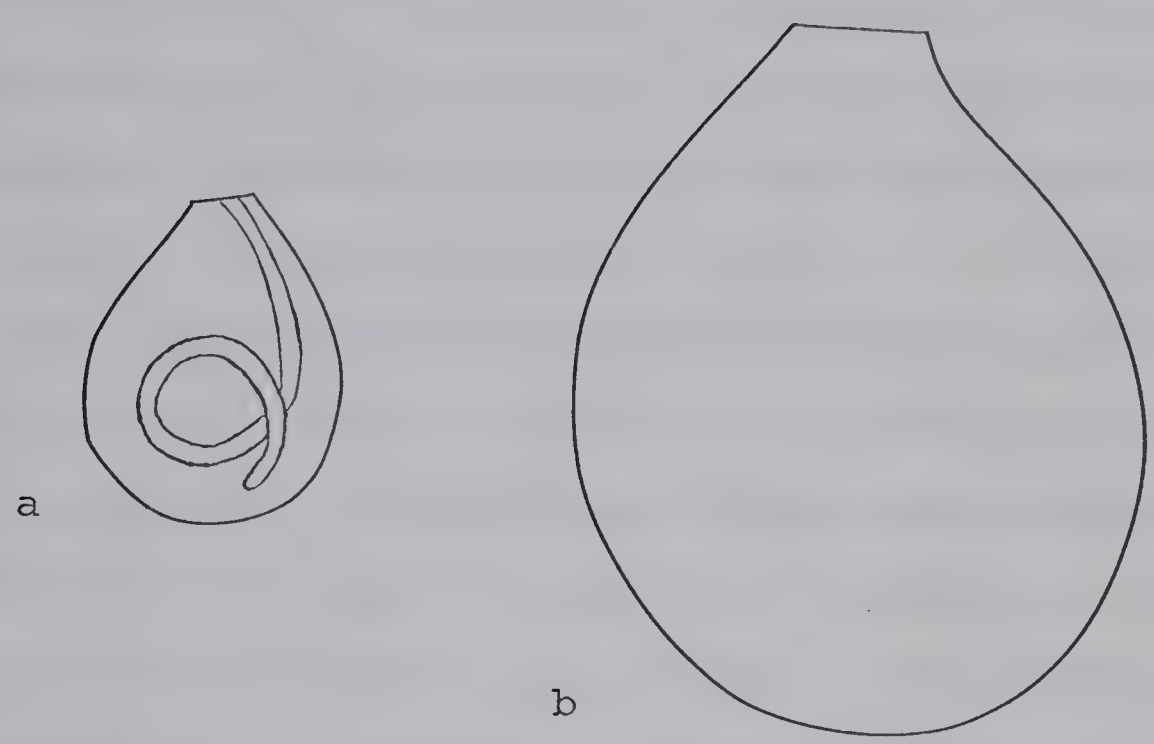

Figure 2. Drawings of the four types of nematocysts of Hydra carnea: (a) desmoneme; (b) stenotele; (c) holotrich; (d) atrich.

providing for exact control of culture medium and food supply. Their method was employed in the present study.

The culture solution consisted of distilled-deionized water containing 10^{-3} M calcium chloride and 10^{-3} M sodium bicarbonate with a pH of 7.5-8.0. Finger bowls, 4.5 inches in diameter, containing culture solution were used to hold the animals. These vessels were placed in a circulating water bath, the temperature of which was maintained at 21±0.5°C with a Blue M Constant-Flow Portable Cooling Unit, Model No. PCC-13A. The culture solution was changed every day to keep it as fresh as possible. In addition, the culture dishes were cleaned once a week to prevent the accumulation of slime. To facilitate this cleaning, the hydra were detached from the glass bowl with a forceful spray of solution from a pipette and transferred to another container. Any tissue damage due to this forceful detachment of the animals is readily repaired with no ill effects to the hydra (Loomis and Lenhoff, 1956).

For feeding the animals, freshly hatched nauplii larvae of brine shrimp, Artemia salina, were used. The dried eggs were sprinkled on a brine solution with a density of 1.04 grams per milliliter and allowed to hatch at room temperature. Under these conditions approximately 48 hours were needed for hatching. The larvae were then removed with a large pipette, separated from the brine solution by filtration, and washed with hydra culture solution. They were

then added to the cultures of hydra with a small pipette. After 30 minutes the dishes were rinsed with fresh culture solution to remove any uningested brine shrimp. The brine shrimp die and decompose rapidly in fresh water and so must be removed as soon as possible. The solution was changed again about six hours after feeding to remove all egested material.

The animals were maintained in light-tight culture chambers made of plywood (Figure 3). Each door provided access to two culture chambers, the fronts of which were covered with black felt. Each chamber measured 13.5 x 18.5 inches with a depth of 15.5 inches. Water, pumped from the constant temperature bath, passed through Tygon tubing into each chamber from the rear and drained out through tubing in the front (Figure 3). The water thus circulated around the culture dishes placed in a plastic dishpan within each chamber.

The hydra were cultured under conditions of constant illumination to avoid any effects of the diurnal rhythm Passano and McCullough (1964) found in the contraction burst frequencies. Sylvania F 20T12-CW (cool white) fluorescent tubes were used as the overhead light source. This light passed through 12-inch square filters to control wavelength before the light reached the hydra cultures. Each filter was placed at a distance of five inches from three fluorescent tubes. CBS filters (Carolina Biological

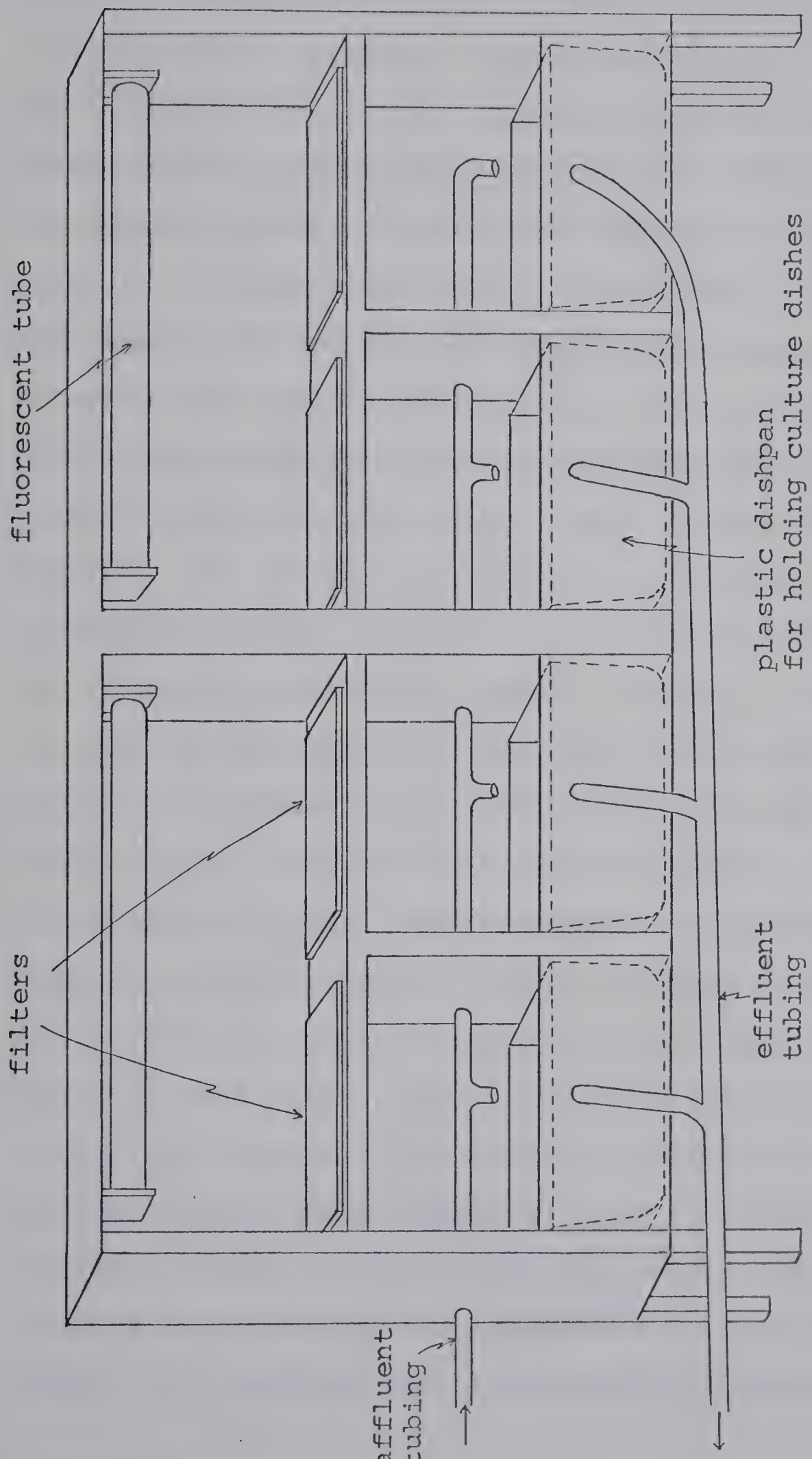

Figure 3. Light-tight chambers used for culturing the hydra in a light-controlled environment. (Doors and felt covers removed.)

Supply Company) with transmission peaks of 450, 545 and 650 nm were used to provide blue, green and red culture lights in three of the chambers. The transmission of these filters was adjusted with neutral density filters to provide all three culture groups with light of equal overall intensity. The neutral density filters were prepared by coating one side of a Plexiglas square with smoke from a benzene-natural gas flame until the desired density was reached and then spraying with clear acrylic resin. The composition of the light transmitted by the three corrected filters, using three fluorescent tubes as the light source, is shown in Figure 4. As can be seen, the filters differ in the energy transmitted at the wavelength of maximum intensity. This was necessary since equal overall intensity, indicated by the area of each peak, was desired, for the blue and red filters transmitted broader peaks than the green filter. Diffuse white light, with an overall intensity equal to each of the other filters, was provided for the fourth culture group by a neutral density filter prepared as described above. Figure 5 shows the composition of the light transmitted by this filter, again using three fluorescent tubes as the light source. The spectral transmission for each of the four filters was measured with an ISCO Model SR Spectroradiometer (Instrumentation Specialties Company, Inc.), the readings of which have been corrected for the variable sensitivity of the instrument throughout the spectrum. The

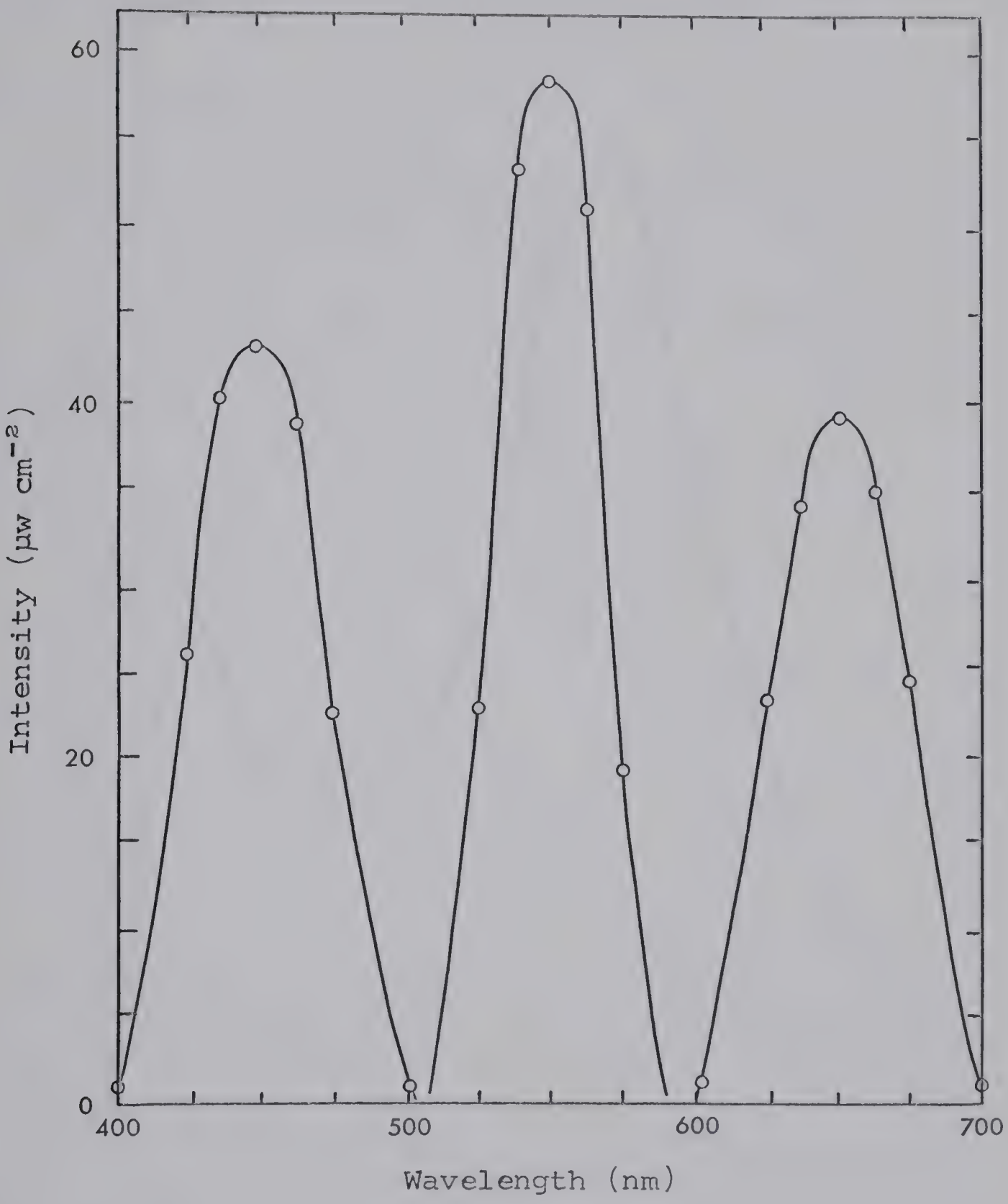

Figure 4. Composition of the light transmitted by the corrected filters used to culture animals under blue, green and red lights, with three fluorescent tubes as the light source.

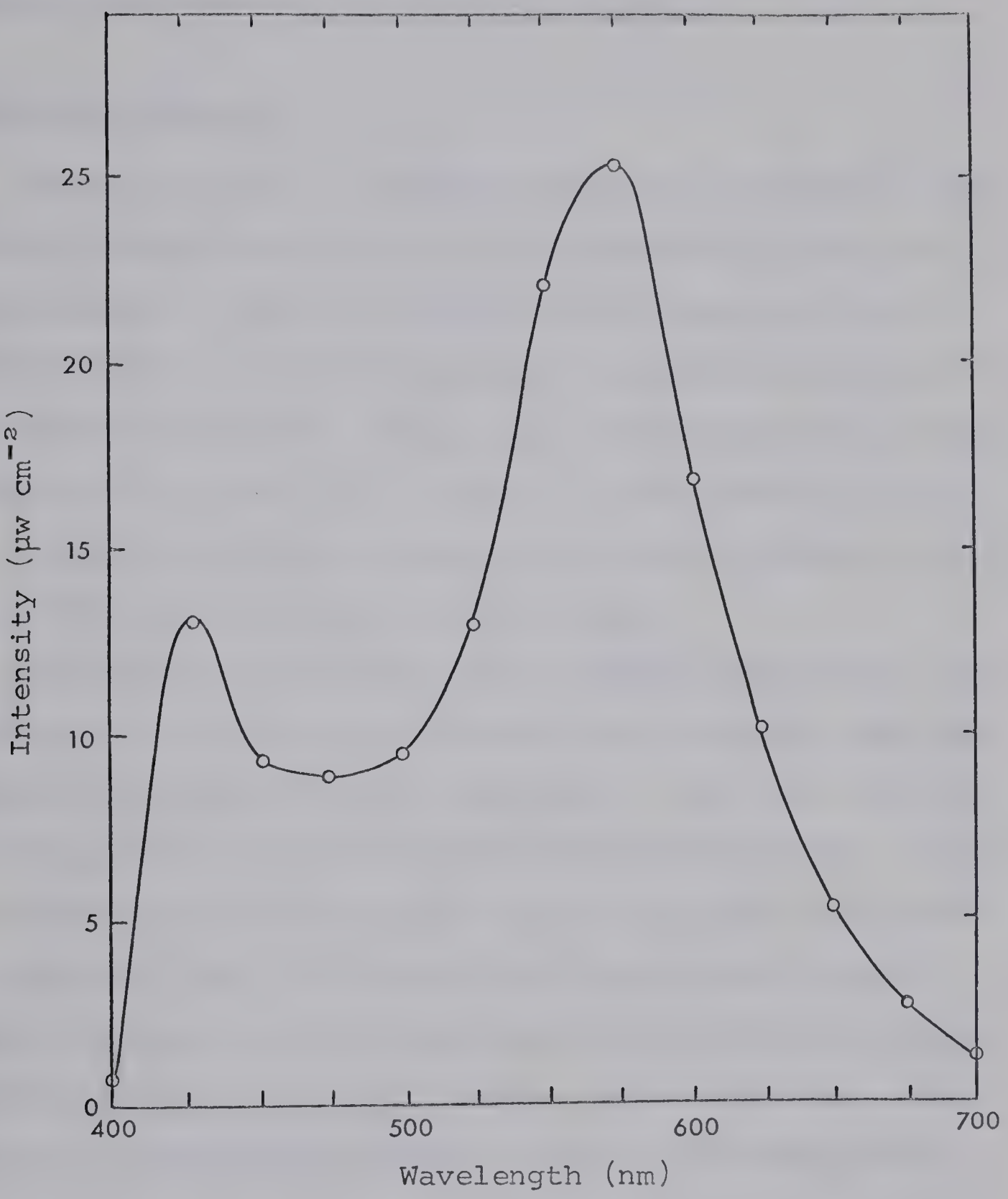

Figure 5. Composition of the light transmitted by the neutral density filter used to culture animals under diffuse white light, with three fluorescent tubes as the light source.

transmission of each filter was measured twice during the course of the study; no change was observed.

Growth Rate Studies

Growth in hydra is characterized by an increase in the number of individuals rather than an increase in the size of each animal. When fed frequently and regularly, the animals undergo asexual reproduction producing additional individuals by budding. Thus, the rate of growth can easily be measured by counting the number of individuals each day over a specific period of time, as was done by Loomis (1953). Such a method was followed in this study.

In measuring the growth rate of Hydra carnea under four conditions of illumination, animals from one clone were used throughout to reduce genetic variables. The clone used had previously been maintained under diffuse white light. Eight individuals were taken from the clone, and each was placed in a separate dish. Two dishes were then placed in each chamber so that two simultaneous growth rate studies could be conducted under each experimental light condition. The studies were started on October 2, 1967, and continued for 40 days. During this time, the animals were fed every morning, and the number of individuals present in each dish were counted prior to feeding each day. The same procedure was repeated for a second set of studies which was initiated on November 20, 1967, using individuals from the same clone as

before. Thus four growth rate studies were conducted under each of the four light conditions to provide growth rate data for animals taken from diffuse white light and placed under the experimental lights.

To determine if the growth rate pattern would change after the animals had been under the experimental lights for a period of time, a third set of studies was initiated on December 30, 1967. The same procedure was followed except that the two animals used to start the studies under each light were taken from the culture which developed during the growth rate studies initiated on October 2, 1967. Thus, the animals used had been exposed to that light for more than two months.

Action Spectrum Studies

Since hydra shows a contraction response to a light stimulus, it is possible to determine the sensitivity of the animal to various wavelengths of light by studying this contraction response as a function of wavelength. There are two possible ways to conduct such a study. First, one may determine the intensity of light necessary at each wavelength to cause a specific response, as was done by North and Pantin (1958) with the sea anemone Metridium senile. They determined the intensity necessary at each wavelength to cause a bending response. Secondly, one may determine how a certain response varies with wavelength,

using light of equal intensity at each wavelength. This was done by Singer, Rushforth and Burnett (1963) with Hydra pirardi, using the length of time between onset of the light stimulus and contraction of the animal as the variable response measured. This second method was employed in the present study.

Figure 6 shows the system used to test an animal's contraction time. The animal to be tested was held in a 45 x 15 mm flat-bottomed vial containing hydra solution to a depth of 2.5 centimeters. Light from the monochromator passed horizontally to a 50 mm plane mirror by which it was reflected down so as to illuminate the animal from above. That part of the apparatus from the exit slit of the monochromator to the vial holding the hydra was enclosed in a light-tight box to eliminate stray room light. An opening for viewing the animal was provided on the side of the box. A shutter was placed between the monochromator and the light source for controlling the onset of the light stimulus during testing.

To provide light of the specific wavelengths desired, a Bausch and Lomb No. 33-86-25-02 High Intensity Monochromator with a visible grating (350-800 nm) was used in conjunction with a Bausch and Lomb Quartz No. 1 Collective Lens and fixed entrance and exit slits with widths of 1.34 millimeters and 0.75 millimeters, respectively. The light was provided by a Bausch and Lomb No. 33-86-20-01 Xenon

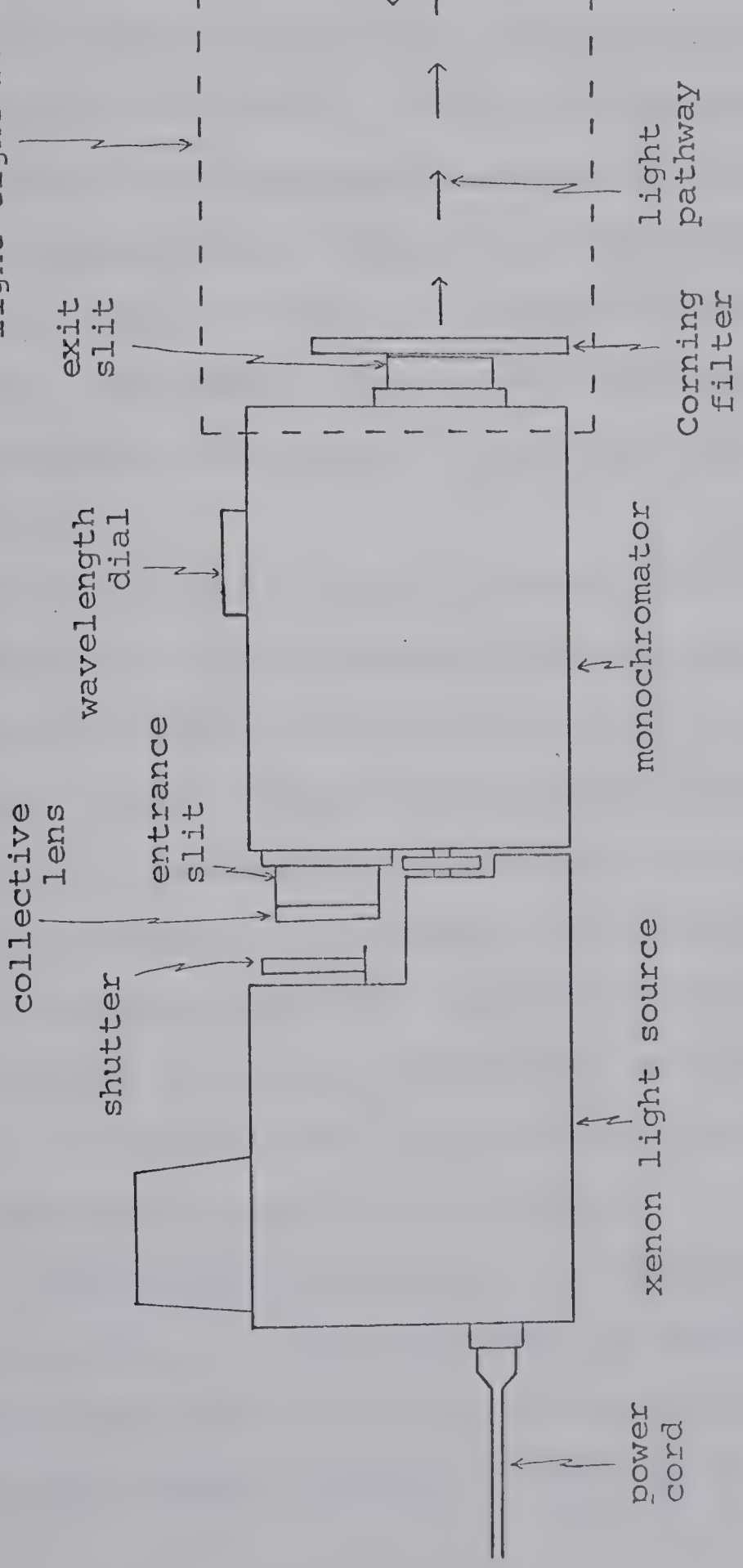

Figure 6. Monochromator system used for testing an animal's contraction time to a light stimulus of a specific wavelength.

Light Source with a constant wattage DC power supply. Corning filters No. 0-54 and No. 3-69 were used at wavelength ranges 350-560 nm and 560-800 nm, respectively, to eliminate overlapping orders of light. The appropriate filter was mounted at the exit slit of the monochromator. The system was calibrated, using the spectroradiometer mentioned earlier, by measuring the radiant output as a function of wavelength at a distance of ten centimeters from the exit slit. Figure 7 shows the spectral output measured for the system. As before, the readings have been corrected for the variable sensitivity of the instrument throughout the spectrum.

To obtain light of equal intensity at all wavelengths, the distance the light traveled from the exit slit of the monochromator to the animal to be tested was varied with wavelength. Since intensity is inversely proportional to the distance squared, this relationship was employed to calculate the distances to be used. The output of the system at these distances was then checked with the spectroradiometer to ensure that equal intensities were obtained at all settings. Table II shows the distance used at each wavelength setting for testing the animals.

The animals were tested at 11 wavelengths, every 25 nm from 425 to 675 nm. For each test, an animal was transferred from the culture dish to a vial containing hydra solution and allowed to adapt in the dark for 30 minutes.

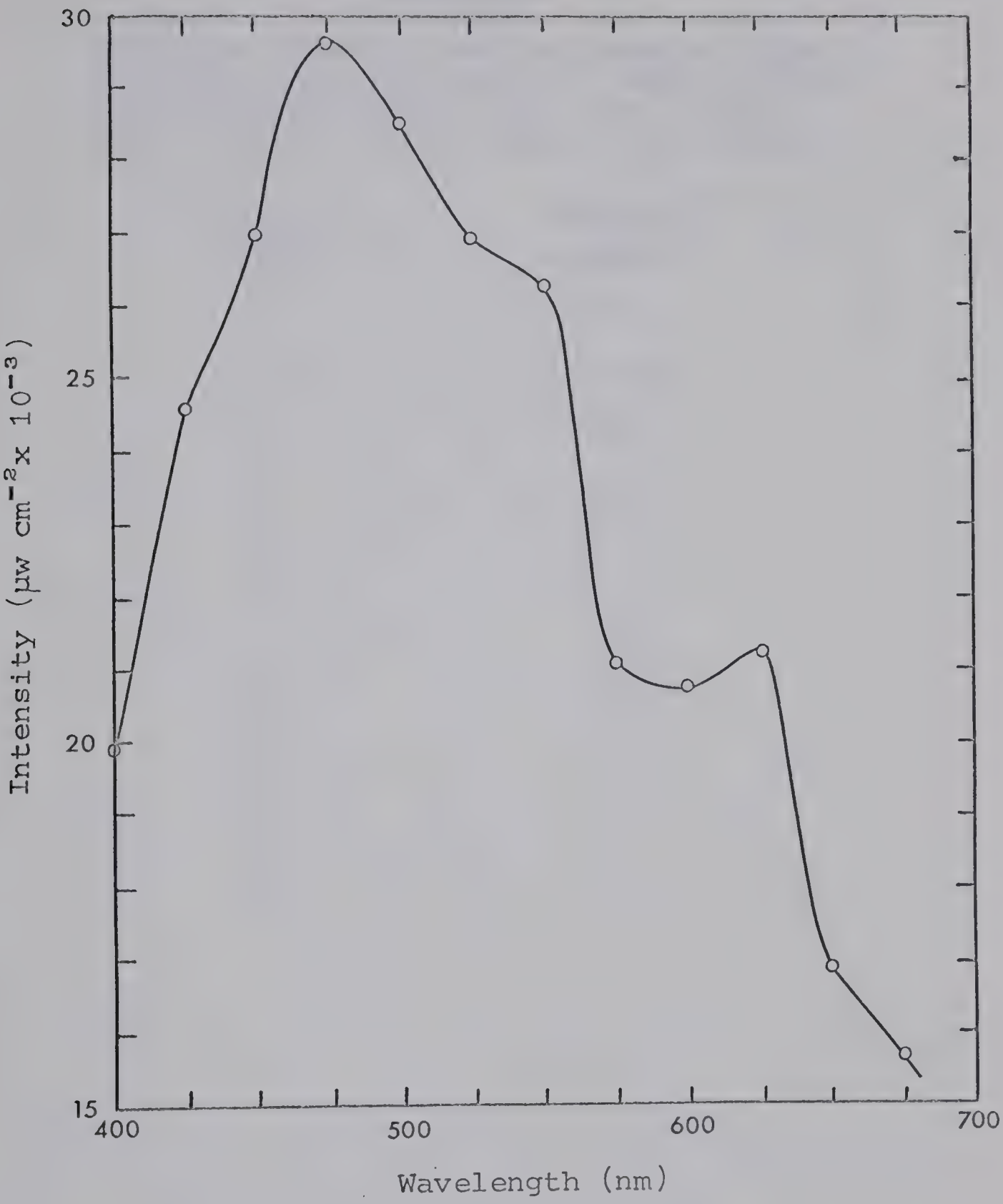

Figure 7. Spectral output of the monochromator using the xenon light source. Measurements were made at a distance of ten centimeters from the exit slit of the monochromator.

Table II

Distances used to test the reaction times of hydra to 11 wavelengths. Each distance was measured from the exit slit of the monochromator to the animal to be tested.

Wavelength (nm)	Distance (cm)
425	8.97
450	9.39
475	9.83
500	9.64
525	9.38
550	9.27
575	8.29
600	8.24
625	8.34
650	7.43
675	7.17

The vial was then placed in position for testing, and after one minute, the animal was subjected to the light stimulus. The reaction time, that time from the onset of the light stimulus to contraction of the animal, was measured in seconds with a stopwatch. To obtain a mean reaction time, 25 animals were tested at each wavelength. This procedure was followed for animals which had been cultured under white, blue, green and red lights for three months in order to obtain a mean reaction time at each wavelength for each of the four culture groups. The hydra used in these tests were from the cultures developed during the growth rate studies initiated on October 2, 1967.

After the above tests had been completed, a group of animals from each light condition was cultured in the dark for one month, with brief exposure to light only when the culture solution was changed daily or when the animals were fed. These animals were then tested again for their sensitivity at three wavelengths: 450, 550 and 650 nm.

To establish a control to show how frequently H. carnea will contract when not subjected to any special light stimuli, the contraction of animals in diffuse room light was observed. Twenty-five animals from the culture under white light were placed in a dish in the laboratory, and the length of time between two contractions was measured for each. The mean of these contraction times was taken as the standard contraction time.

III. RESULTS

Growth Rate Studies

The results of the growth rate studies under white, blue, green and red lights are summarized in Table III.

Table III

Asexual growth of *Hydra carnea* under white, blue, green and red lights. Each value is the total number of individuals for six studies under each light.

Day	Culture Light			
	White	Blue	Green	Red
1	6	6	6	6
10	20	17	18	23
20	68	53	55	134
30	126	104	123	348
40	326	182	297	768

More complete data are presented in Appendix I. In this appendix, the number of individuals every fifth day for each study is tabulated, as well as a total every fifth day of the six studies of each culture group.

Loomis (1953) has shown that the growth rate of hydra was logarithmic; a plot of the logarithm of the number of individuals each day *vs.* the day of the study should be linear. Therefore, a linear least-squares analysis, the

computer program for which is found in Appendix II, was performed on the data of each study to yield a best-fit value for the slope of each line. Days 6 through 40 were used in this analysis; the first five days of each growth rate study were arbitrarily deleted from the analysis to eliminate any effects of an initial period of adjustment which may have occurred due to the disturbance of the animals at the initiation of each study. Table IV shows the slopes obtained from these analyses.

By analysis of the six growth rate studies under each experimental light condition, it can be seen that the growth rate of animals taken from diffuse white light and placed under blue, green or red light (studies 1-4) did not differ significantly from the growth rate of animals maintained under that same experimental light for two months (studies 5-6). In Table V are presented the averages of the slopes of the growth rate curves under these two conditions for comparison to verify this conclusion. Animals cultured under diffuse white light throughout this time showed no change in their growth rate; the average of the growth rate slopes during the time of the first four studies was 4.11 ± 0.16, while the average during the last two studies was 4.10 ± 0.16. Since the growth rate of the animals under diffuse white light did not change, one can assume that there was no alteration in the culturing conditions during this time.

Table IV

Values obtained by linear least-squares analysis for the slopes of the semilogarithmic plots of the growth rate data.

Study Number	Culture Group White	Blue
1	4.59±0.16	4.17±0.21
2	3.71±0.11	3.78±0.21
3	4.65±0.15	3.76±0.22
4	3.48±0.16	3.42±0.15
5	3.77±0.16	3.54±0.18
6	4.43±0.10	4.10±0.16
total	4.01±0.09	3.72±0.17

Study Number	Culture Group Green	Red
1	4.51±0.18	5.75±0.24
2	4.37±0.19	4.68±0.11
3	4.16±0.10	5.59±0.28
4	3.74±0.15	5.15±0.10
5	4.14±0.18	5.91±0.31
6	4.28±0.22	4.67±0.22
total	4.15±0.12	5.18±0.18

Table V

Average values for the slopes of the semi-logarithmic plots of the growth rate data for animals with no previous exposure and with one month exposure to the experimental light

Experimental Light	No Previous Exposure to the Experimental Light (Aver. of 4 Studies)	One Month Prior Exposure to the Experimental Light (Aver. of 2 Studies)
Blue	3.78±0.22	3.82±0.18
Green	4.20±0.19	4.21±0.22
Red	5.29±0.31	5.29±0.28

In Figures 8, 9, 10 and 11, semilogarithmic plots of the growth rate data for each of the four culture groups are presented, showing the logarithm of the total number of individuals every second day from the six combined studies for each gorup vs. the day of the study. The total number of individuals is used for comparison of the four culture groups because it provides for increased statistical validity by using a larger sample. As can be seen from the figures, the animals cultured under blue light had the slowest growth rate, with a slope of 3.72±0.17. Animals cultured under red light had the greatest growth rate, with a slope of 5.18±0.18. The slope of the curve for animals cultured under white light (4.01±0.09) is most similar to that for animals cultured under green light (4.15±0.12) because the white light used in this experiment had its maximum

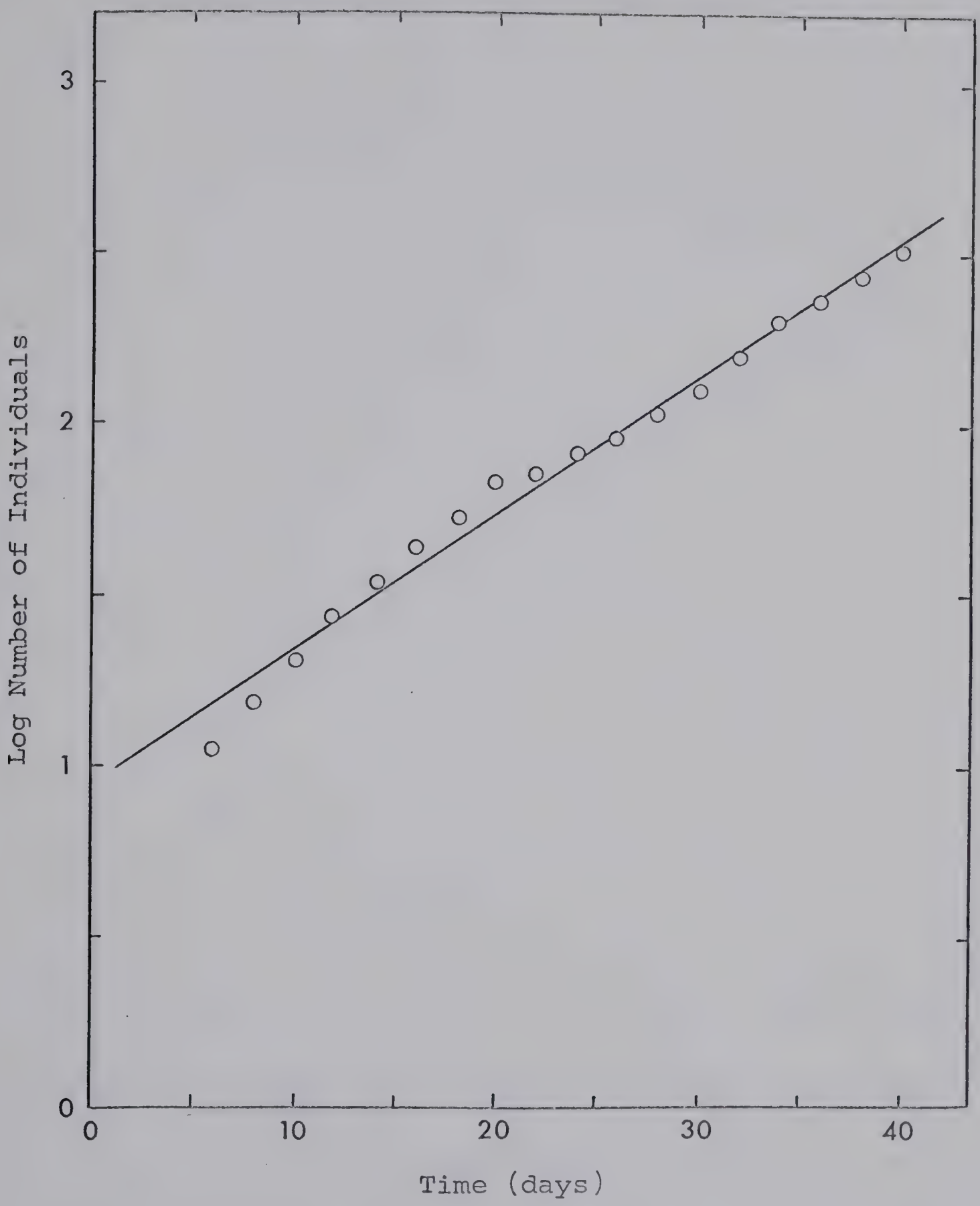

Figure 8. Semilogarithmic plot of the growth rate data obtained for animals cultured under white light. Line shows the best-fit value for the slope as determined by linear least-squares analysis of the data.

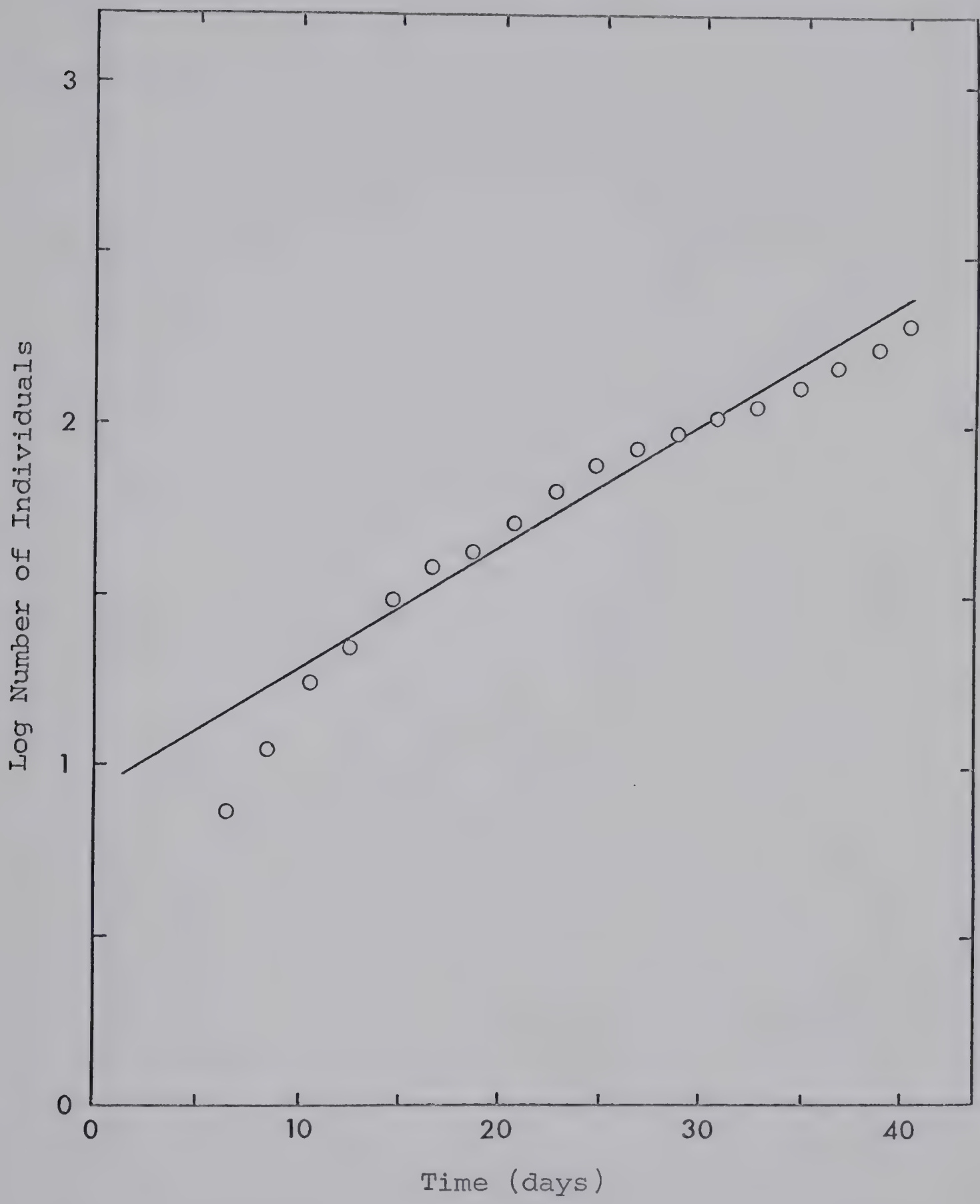

Figure 9. Semilogarithmic plot of the growth rate data obtained for animals cultured under blue light. Line shows the best-fit value for the slope as determined by linear least-squares analysis of the data.

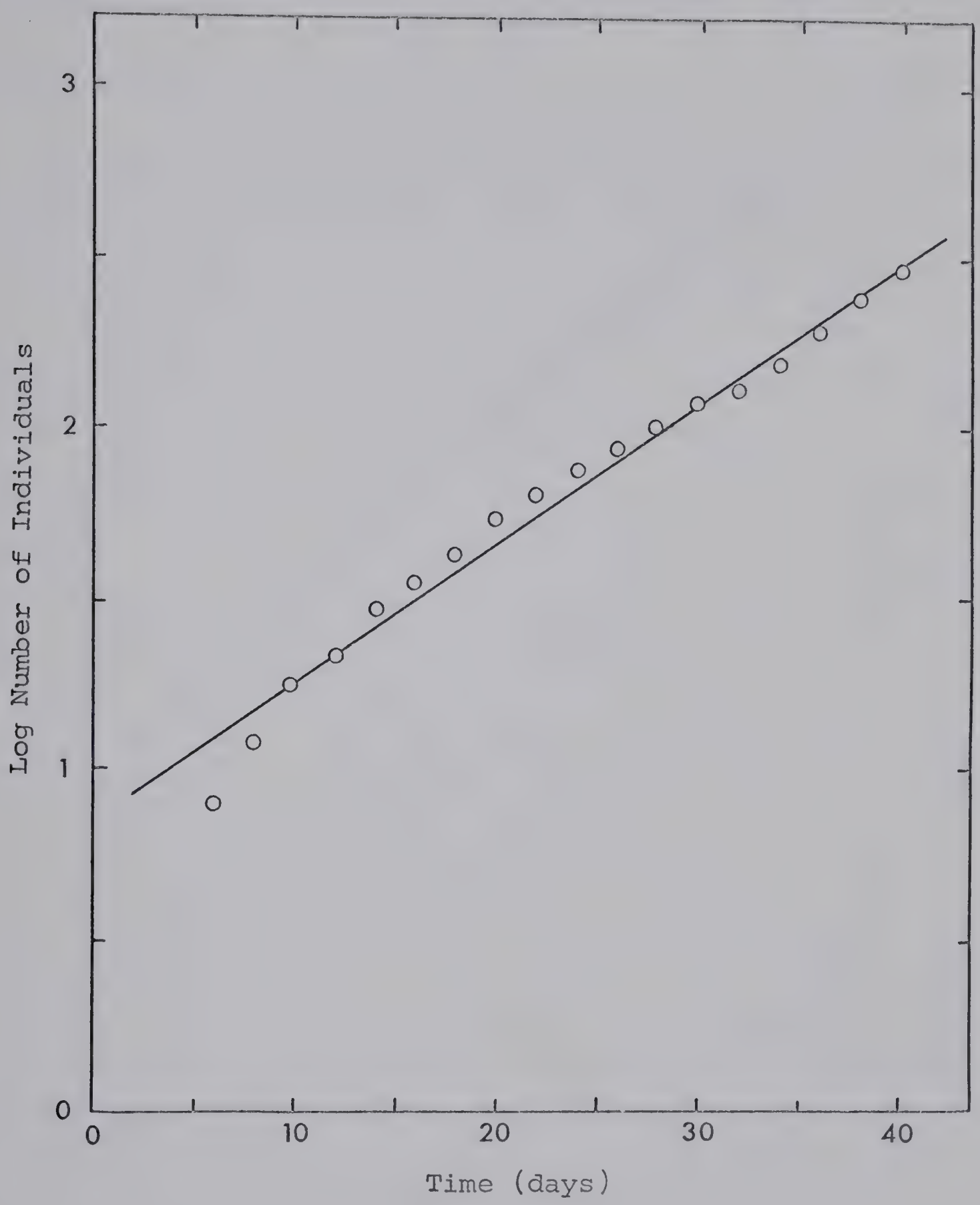

Figure 10. Semilogarithmic plot of the growth rate data obtained for animals cultured under green light. Line shows the best-fit value for the slope as determined by linear least-squares analysis of the data.

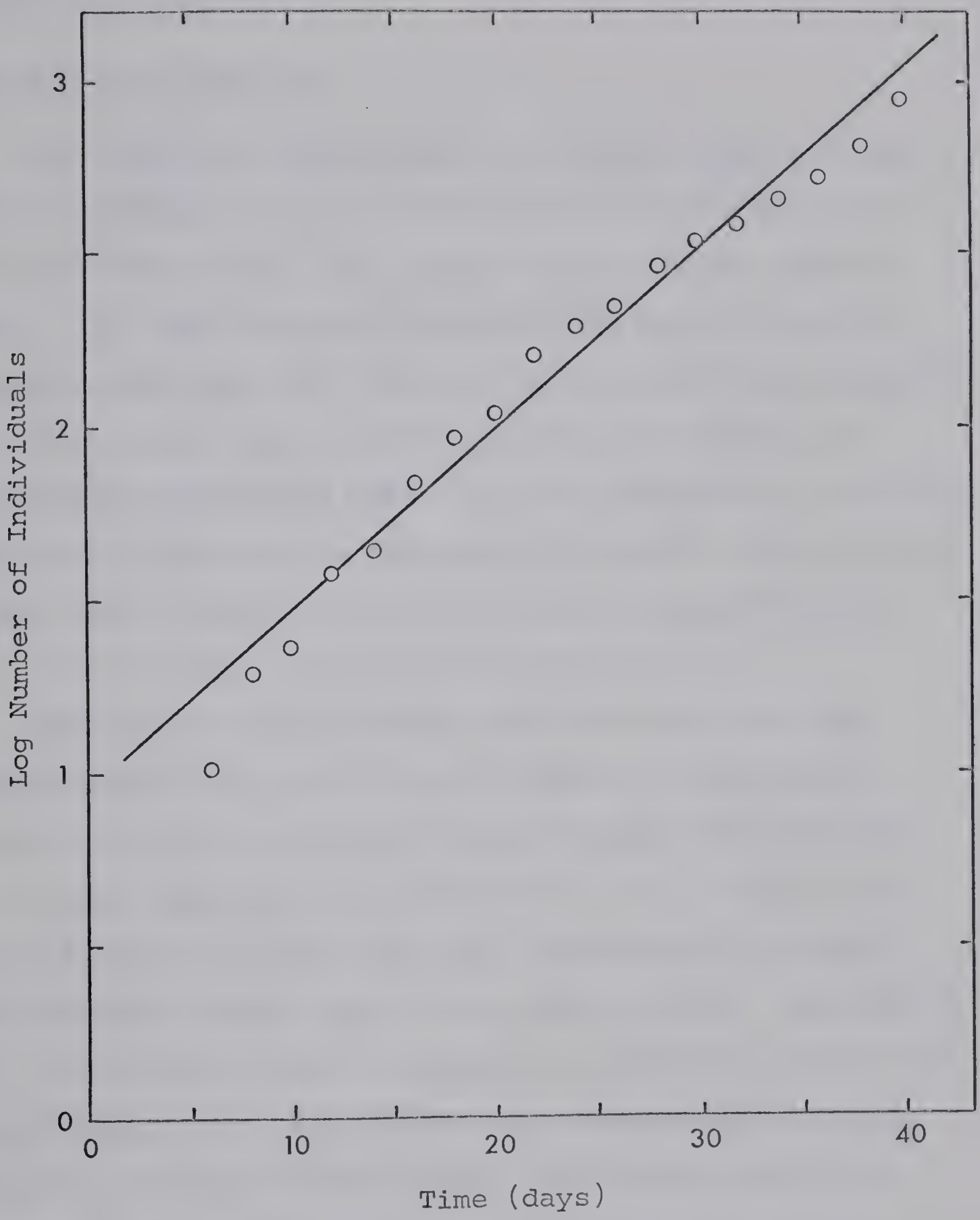

Figure 11. Semilogarithmic plot of the growth rate data obtained for animals cultured under red light. Line shows the best-fit value for the slope as determined by linear least-squares analysis of the data.

intensity in the green.

Action Spectrum Studies

The results of the studies on reaction times are presented in Tables VI, VII, VIII and IX for the individuals cultured under white, blue, green and red light, respectively. In each table are tabulated the mean, standard deviation and range for the reaction times of the respective culture group at each of the 11 wavelength settings at which measurements were made. The data obtained for animals cultured in the dark are presented in Table X. The computer program used to find the mean and standard deviation for each set of values is given in Appendix III.

Since an animal's reaction time increases with decreased sensitivity to the light stimulus, the relative sensitvity can be determined by calculating the recirocal of the mean reaction time. Figures 12, 13, 14 and 15 are plots of relative sensitivity vs. wavelength for animals cultured under white, blue, green and red light, respectively. Figures 16, 17 and 18 present the relative sensitivity to wavelengths 450, 550 and 650 nm, respectively, of animals from the four culture groups after having been cultured in the dark for one month.

Analyses of the reaction times of animals cultured under different light conditions are made quantitative by use of the Student's "t" test, the results of which are

Table VI

Reaction times to light of various wavelengths for hydra cultured under white light. Twenty-five animals were tested at each wavelength.

Wavelength (nm)	Reaction Time (seconds) Mean	Std Dev	Range
425	267	48	175-399
450	275	33	216-320
475	223	51	121-309
500	249	43	186-337
525	241	33	180-302
550	307	65	206-437
575	156	22	112-201
600	105	19	75-134
625	88	20	56-126
650	93	23	53-145
675	150	34	99-219

Table VII

Reaction times to light of various wavelengths for hydra cultured under blue light. Twenty-five animals were tested at each wavelength.

Wavelength (nm)	Reaction Time (seconds) Mean	Std Dev	Range
425	274	40	220-363
450	348	43	272-426
475	302	47	240-398
500	229	51	137-339
525	208	34	124-302
550	214	50	99-348
575	258	51	181-361
600	303	47	216-372
625	160	30	100-208
650	114	24	66-179
675	151	46	65-253

Table VIII

Reaction times to light of various wavelengths for hydra cultured under green light. Twenty-five animals were tested at each wavelength.

Wavelength (nm)	Reaction Time (seconds) Mean	Std Dev	Range
425	197	49	132-304
450	241	38	171-317
475	203	42	119-284
500	219	50	140-344
525	318	48	222-432
550	385	55	283-468
575	256	49	115-333
600	133	47	77-261
625	95	20	64-136
650	112	30	65-196
675	138	34	84-204

Table IX

Reaction times to light of various wavelengths for hydra cultured under red light. Twenty-five animals were tested at each wavelength.

Wavelength (nm)	Reaction Time (seconds) Mean	Std Dev	Range
425	264	42	187-339
450	254	55	163-359
475	252	43	174-321
500	244	39	181-311
525	260	51	188-338
550	213	34	150-274
575	232	51	154-324
600	203	30	136-248
625	193	34	131-248
650	233	39	151-283
675	187	28	141-230

Table X

Reaction times to light of various wavelengths for hydra cultured in the dark. Twenty-five animals from each culture group were tested at each wavelength.

Previous Light Experience	Wavelength (nm)	Reaction Time (seconds) Mean	Std Dev	Range
White	450	274	46	184-354
	550	240	39	142-317
	650	143	32	74-205
Blue	450	286	46	193-385
	550	249	43	164-328
	650	135	27	88-187
Green	450	282	40	211-359
	550	254	50	162-342
	650	136	32	78-197
Red	450	273	46	184-374
	550	246	38	179-316
	650	137	32	76-205

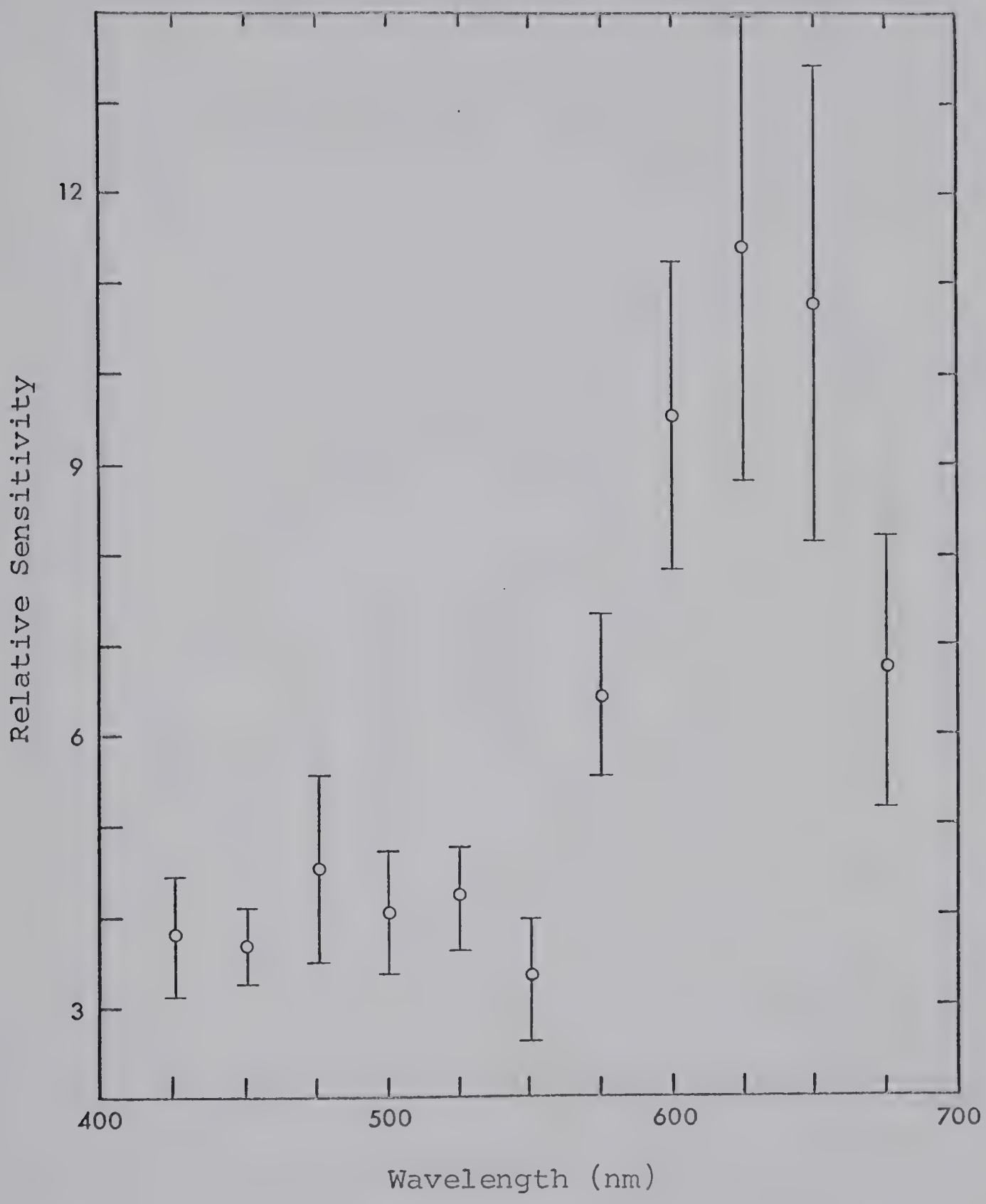

Figure 12. Relative sensitivity *vs.* wavelength for hydra cultured under white light. The relative sensitivity equals 10^3 times the reciprocal of the mean reaction time for 25 animals. The vertical line represents one standard deviation.

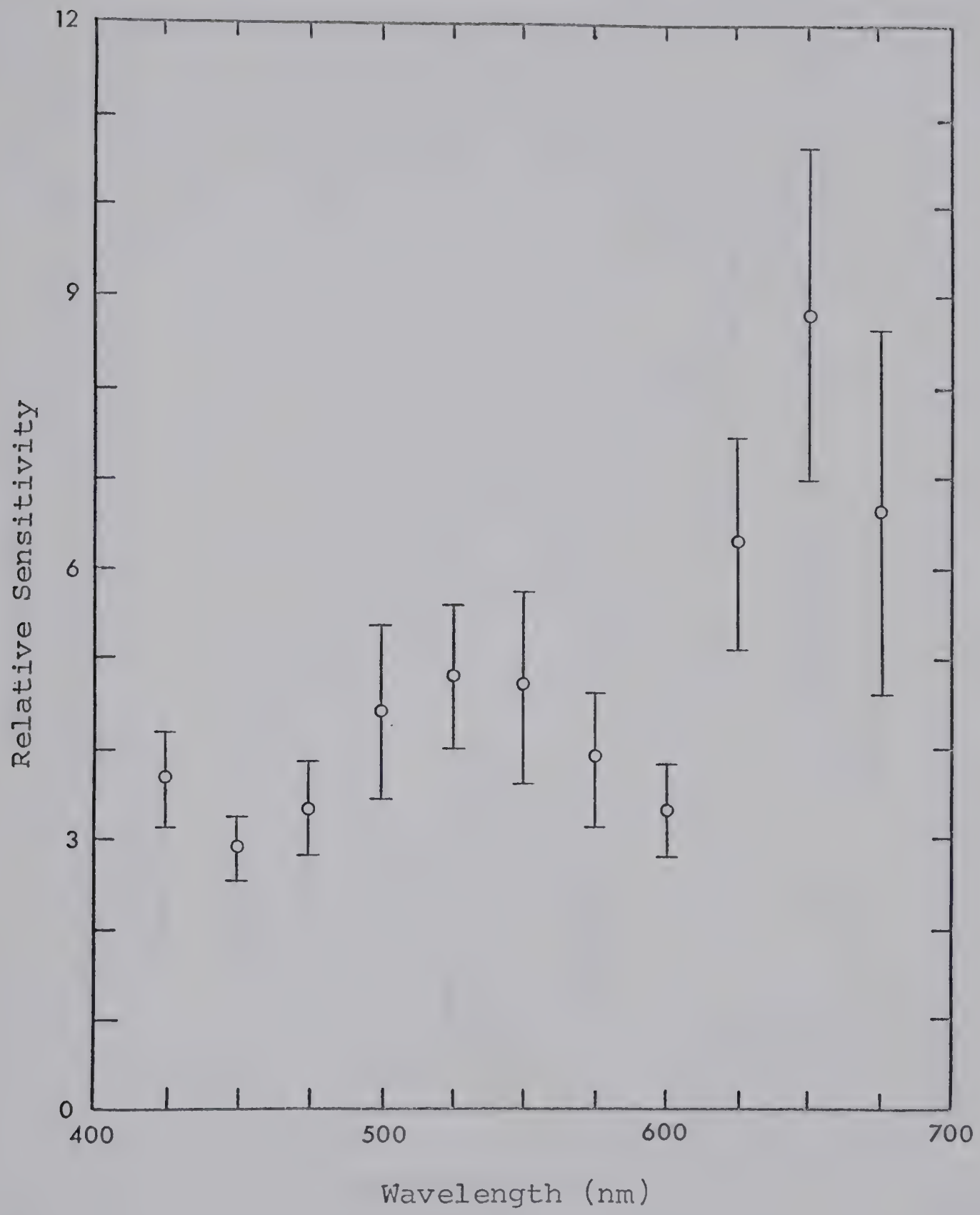

Figure 13. Relative sensitivity *vs.* wavelength for hydra cultured under blue light. The relative sensitivity equals 10^3 times the reciprocal of the mean reaction time for 25 animals. The vertical line represents one standard deviation.

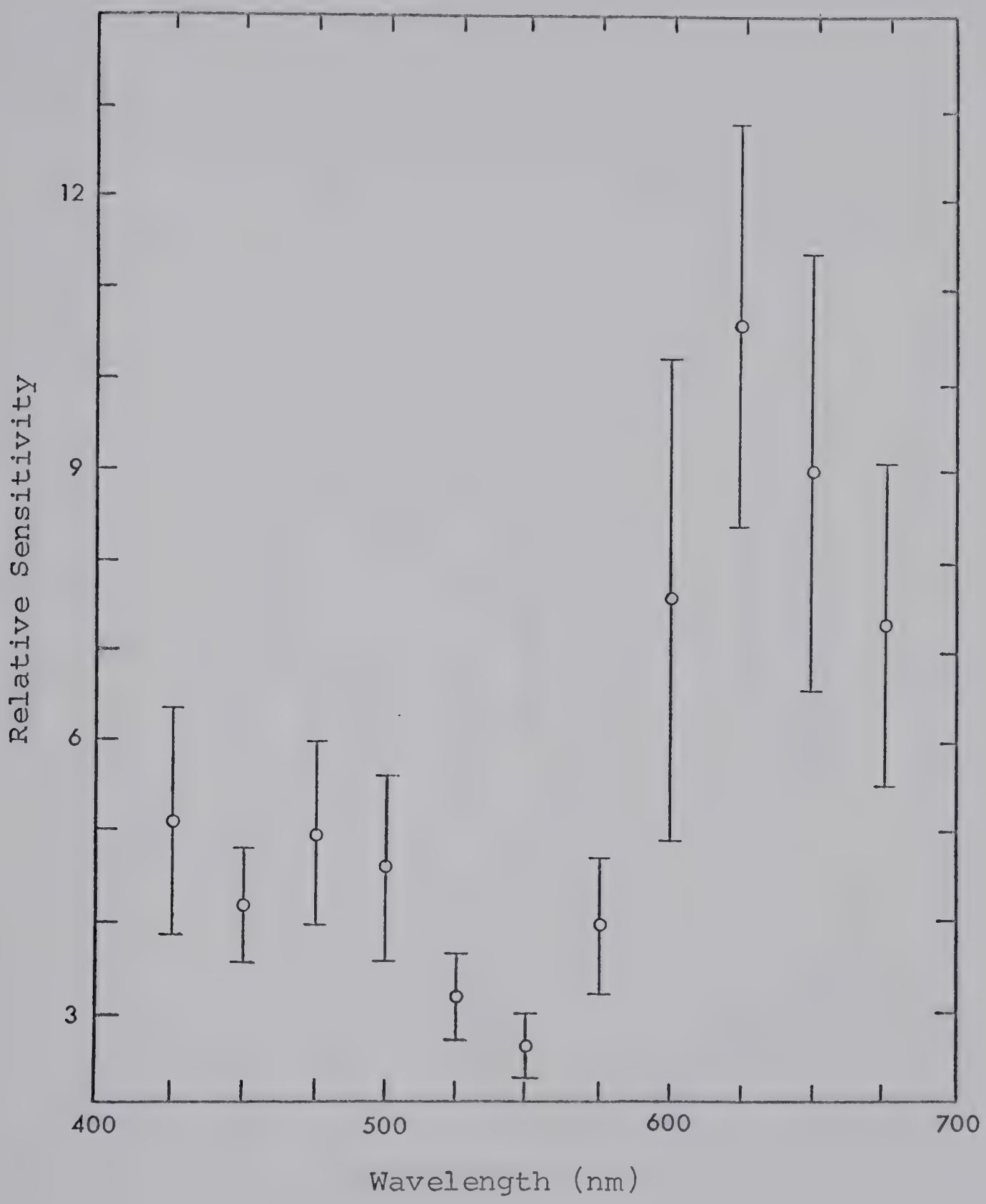

Figure 14. Relative sensitivity *vs.* wavelength for hydra cultured under green light. The relative sensitivity equals 10^3 times the reciprocal of the mean reaction time for 25 animals. The vertical line represents one standard deviation.

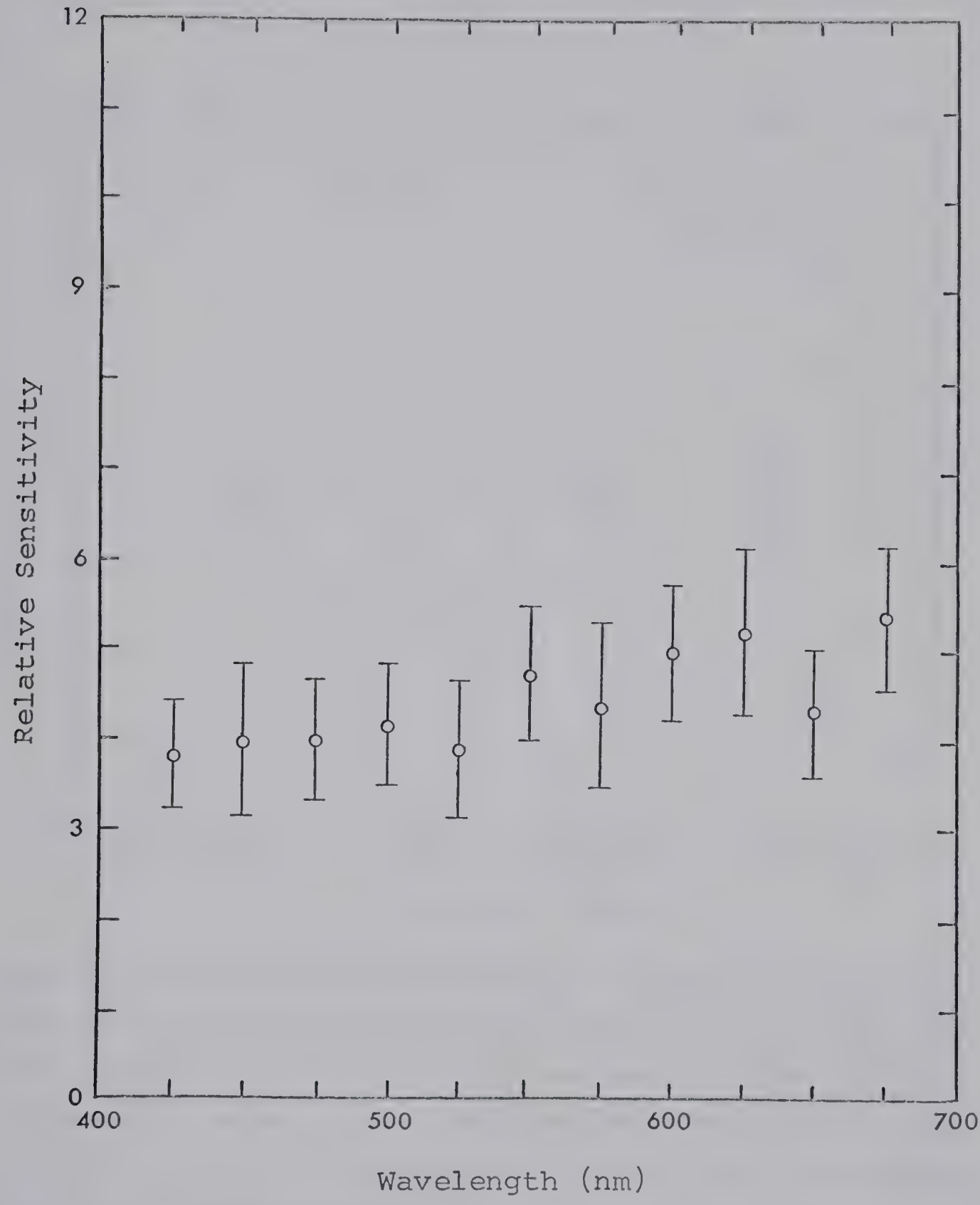

Figure 15. Relative sensitivity *vs.* wavelength for hydra cultured under red light. The relative sensitivity equals 10^3 times the reciprocal of the mean reaction time for 25 animals. The vertical line represents one standard deviation.

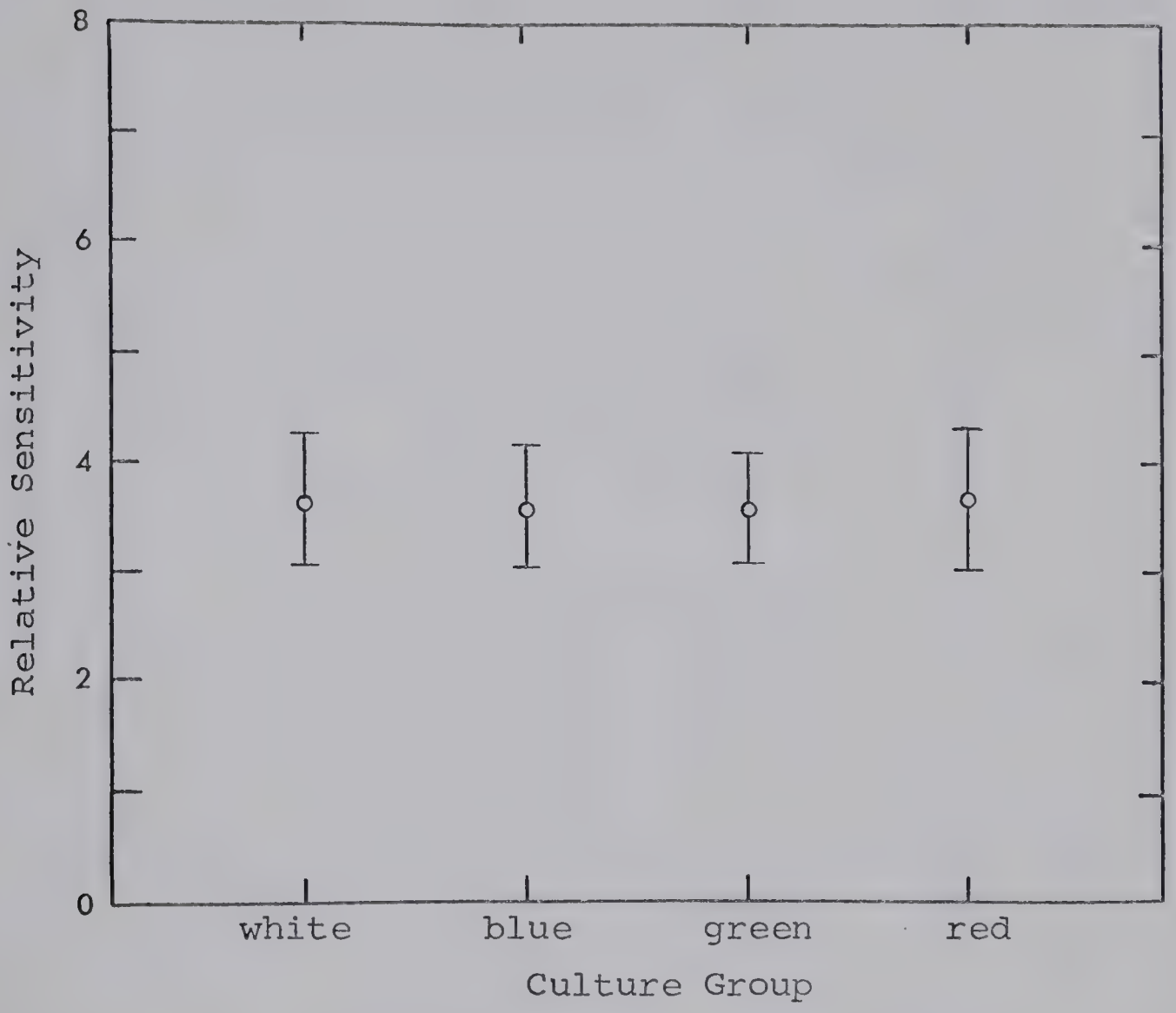

Figure 16. Relative sensitivity to light of wavelength 450 nm for hydra from the four culture groups that have been cultured in the dark for one month. The relative sensitivity equals 10^3 times the reciprocal of the mean reaction time for 25 animals. The vertical line represents one standard deviation.

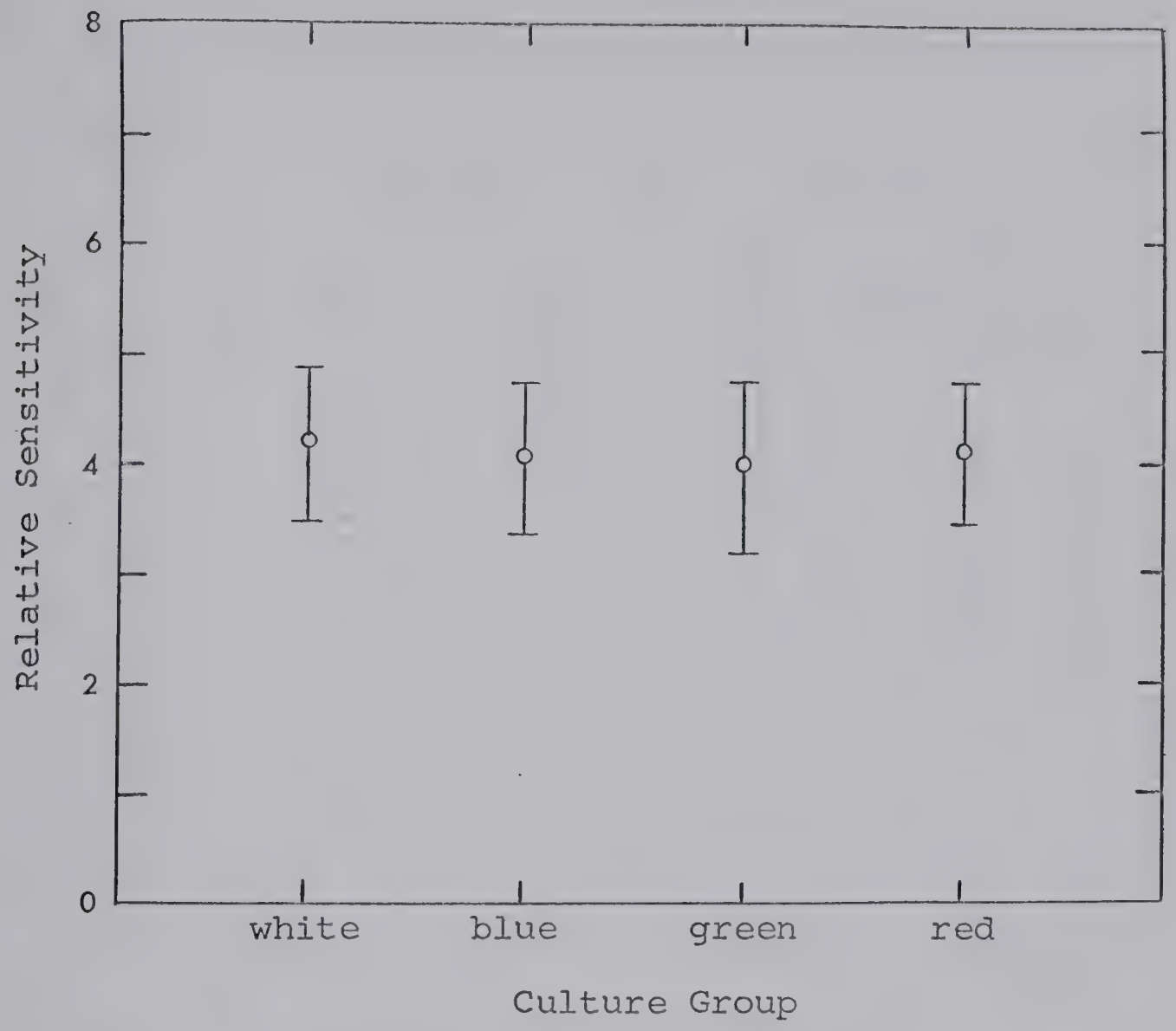

Figure 17. Relative sensitivity to light of wavelength 550 nm for hydra from the four culture groups that have been cultured in the dark for one month. The relative sensitivity equals 10^3 times the reciprocal of the mean reaction time for 25 animals. The vertical line represents one standard deviation.

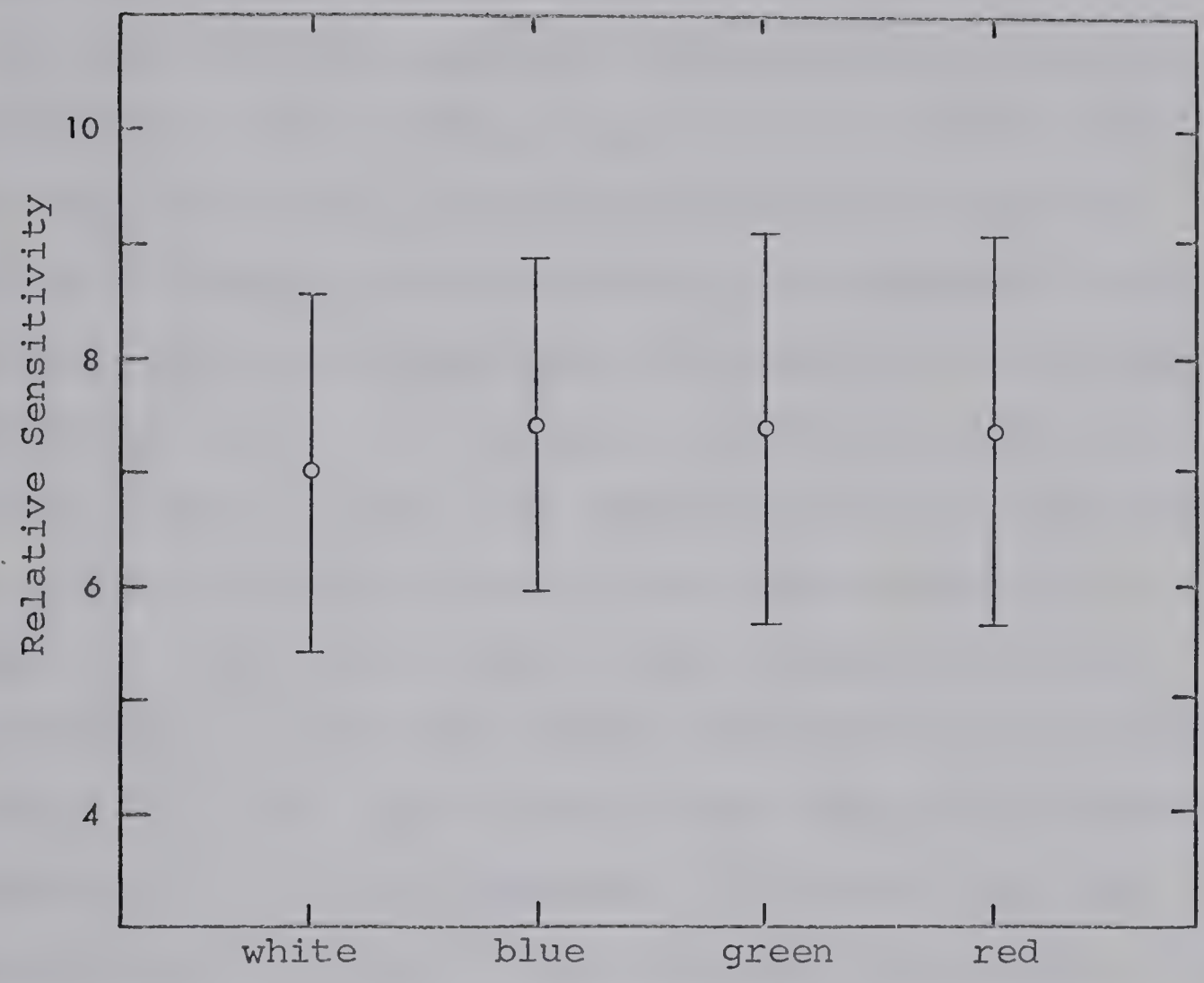

Figure 18. Relative sensitivity to light of wavelength 650 nm for hydra from the four culture groups that have been cultured in the dark for one month. The relative sensitivity equals 10^3 times the reciprocal of the mean reaction time for 25 animals. The vertical line represents one standard deviation.

given in Appendix IV. In the following text, each probability (derived from the "t" test) is the probability that the two means being compared are from the same population.

In comparing the graphs of relative sensitivity vs. wavelength for hydra cultured under the four light conditions (Figures 12-15), it is easily seen that there were definite differences in sensitivity, as measured by reaction time, when the animals were cultured under different experimental lights. For animals that had been cultured in the dark (Figures 16-18), the sensitivity of all four culture groups was equal at each of the three wavelengths tested, for there was no significant difference in the reaction times at each wavelength. Thus the animals showed the same wavelength sensitivities when cultured in identical environments, i.e., when cultured in the dark, but when placed in different environments (white, blue, green and red lights) these same animals varied in their wavelength sensitivities according to their culture light. These changes can best be analyzed by first studying the results obtained with animals cultured in the dark, and then comparing these to the results obtained with animals cultured under each of the four experimental lights.

When the animals were cultured in the dark, they were most sensitive to red light (Figure 18) and least sensitive to blue (Figure 16). In comparing these results to those of the growth rate studies, it can be seen that the animals

increased in number at the greatest rate when cultured under that light to which they were most sensitive (650 nm) in terms of the mean reaction time of animals cultured in the dark; the hydra showed the slowest rate of growth under the light to which they were least sensitive (450 nm) in terms of reaction time.

The animals that were cultured under blue light (Table VII) showed a significant increase in their reaction time to 450 nm ($p<0.001$) and a decrease in their reaction times to 550 nm ($p<0.02$) and 650 nm ($p<0.01$), when compared to animals cultured in the dark (Table X). Animals cultured under green light (Table VIII) had a significantly increased reaction time to 550 nm ($p<0.001$) and decreased reaction times to 450 nm ($p<0.001$) and 650 nm ($p<0.01$) in comparison to animals cultured in the dark. Likewise, animals under red light (Table IX) showed an increased reaction time to 650 nm ($p<0.001$) and decreased reaction times to 450 nm ($p<0.2$) and 550 nm ($p<0.01$). Thus, each of these three culture groups showed a decreased sensitivity, indicated by an increased reaction time, to that light to which they were exposed in the culture chambers. They also showed an increased sensitivity to wavelengths other than that of the culture light when compared to animals cultured in the dark.

At each of the three wavelengths, there was no significant difference ($p<0.001$) in the sensitivities of the two

culture groups not exposed to that particular wavelength in their environment, i.e., there was no significant difference in the reaction times to 450 nm for animals cultured under green and red lights (Tables VIII and IX). Likewise, there was no significant difference in the reaction times to 550 nm and 650 nm for animals cultured under blue and red lights (Tables VII and IX) and under blue and green lights (Tables VII and VIII), respectively. In addition, each pair was significantly higher in sensitivity ($p<0.001$), as indicated by a shorter reaction time, than the group cultured under light of that particular wavelength.

Measurements of the standard contraction time of animals placed in diffuse room light, as a control to show how frequently hydra will contract when not subjected to any special light stimuli, yielded a mean contraction time of 1053 ± 274 seconds with a range of 604 - 1499 seconds. This mean contraction time is well beyond the mean contraction times obtained for animals exposed to the experimental light stimuli.

IV. DISCUSSION

As was indicated in the previous chapter, the growth rate patterns of animals cultured under blue, green and red lights did not change after extended exposure to these environmental conditions. Therefore, it can be postulated that during a period of two months the hydra did not adapt to the new light conditions by any physiological processes which would alter their growth rate patterns. The effect of an experimental light on growth rate was initiated immediately upon exposure to that light.

It has also been shown that the growth rate of *Hydra carnea* was greatest under red light and slowest when cultured under blue light. Therefore, it appears that red light enhanced the growth rate more than green or blue lights did. Since all the culture lights were of equal overall intensity, the total amount of energy reaching the hydra under all four lights was equal. Thus, it does not appear that the amount of energy, *per se*, was the controlling factor affecting the growth rates of the animals. The important factor may have been one which varies with wavelength. One property of light which does vary with wavelength is the quantum energy (E), which varies according to Equation 1:

$$E = \frac{hc}{\lambda} \qquad (1)$$

where h is Planck's constant, c is the velocity of light, and λ is the wavelength. From Equation 1 can be derived the relationship between total light energy (E_T) and the number of quanta (n):

$$E_T = \frac{nhc}{\lambda} \qquad (2)$$

Thus:

$$n = \frac{E_T\lambda}{hc} \qquad (3)$$

If the number of quanta was affecting the growth rates at the different wavelengths, one would expect that the growth rate slope divided by the number of quanta would be constant for these studies. This relationship is shown by Equation 4:

$$\frac{s}{n} = \frac{shc}{E_T\lambda} \qquad (4)$$

where s is the slope of the growth rate curve. The values calculated for this contant (s/n) for animals cultured under blue, green and red lights are:

$$\left(\frac{s}{n}\right)_{450} = (0.66\pm0.03) \times 10^{-20}$$

$$\left(\frac{s}{n}\right)_{545} = (0.61\pm0.02) \times 10^{-20}$$

$$\left(\frac{s}{n}\right)_{650} = (0.63\pm0.02) \times 10^{-20}$$

These values are equal within the bounds of experimental error. Therefore, it appears that the growth rate of H. carnea was dependent on the number of quanta rather than the total light energy.

Since the growth rates of the hydra show a definite relationship to the number of quanta of light rather than the total light energy, the sensitivity as measured by reaction time should also be studied in these terms. If the number of quanta did affect the reaction times, one would expect that the relative sensitivity divided by the number of quanta would be constant. From Equation 3, one can derive a statement of this relationship:

$$\frac{S}{n} = \frac{hc}{tE_{\tau}\lambda} \qquad (5)$$

where S is the relative sensitivity and t is the reaction time. The values calculated for this constant (S/n), using the average of the mean reaction times of animals cultured in the dark for each of the three wavelengths, are:

$$\left(\frac{S}{n}\right)_{450} = (3.5 \pm 0.6) \times 10^{-25}$$

$$\left(\frac{S}{n}\right)_{550} = (3.2 \pm 0.6) \times 10^{-25}$$

$$\left(\frac{S}{n}\right)_{650} = (4.9 \pm 1.1) \times 10^{-25}$$

Although these values are not in as close agreement as those obtained from analysis of the growth rate studies, it does appear that there is a reasonable correlation between the sensitivity of H. carnea, as measured by reaction time, and the number of quanta.

A search of the literature has failed to reveal similar studies on other animals in which growth or reactivity was evaluated on a quantum basis. Hughes (1965) did study

growth in a plant, Impatiens parviflora, on this basis. He found that the efficiency of growth in I. parviflora was greatest in red and least in blue light when evaluated on an energy basis, but on a quantum basis the efficiency of growth was equal in both lights. On a quantum basis, growth efficiency was low in daylight-white light, which has a high green content, probably due to the fact that the plants absorb little green light. In comparison to growth at 15°C in blue-white light, the values obtained by Hughes for relative growth efficiency were:

Light	Energy Basis	Quantum Basis
blue-white	1.00	1.00
blue	0.81	0.96
red	1.19	0.98
daylight-white (green)	0.89	0.89

The results obtained by Hughes compare favorably with the results of the present study. In both cases, blue light was less efficient on an energy basis because there is higher energy per quantum at shorter wavelengths.

The growth rate of Hydra carnea cultured under white light can be compared with the rates obtained by Loomis (1953) for somatic growth in H. littoralis and Chlorohydra viridissima. Figure 19 shows the growth rates for these three species. For Hydra carnea, the days plotted as 1-5 in this figure are actually days 5-9 of the study. No

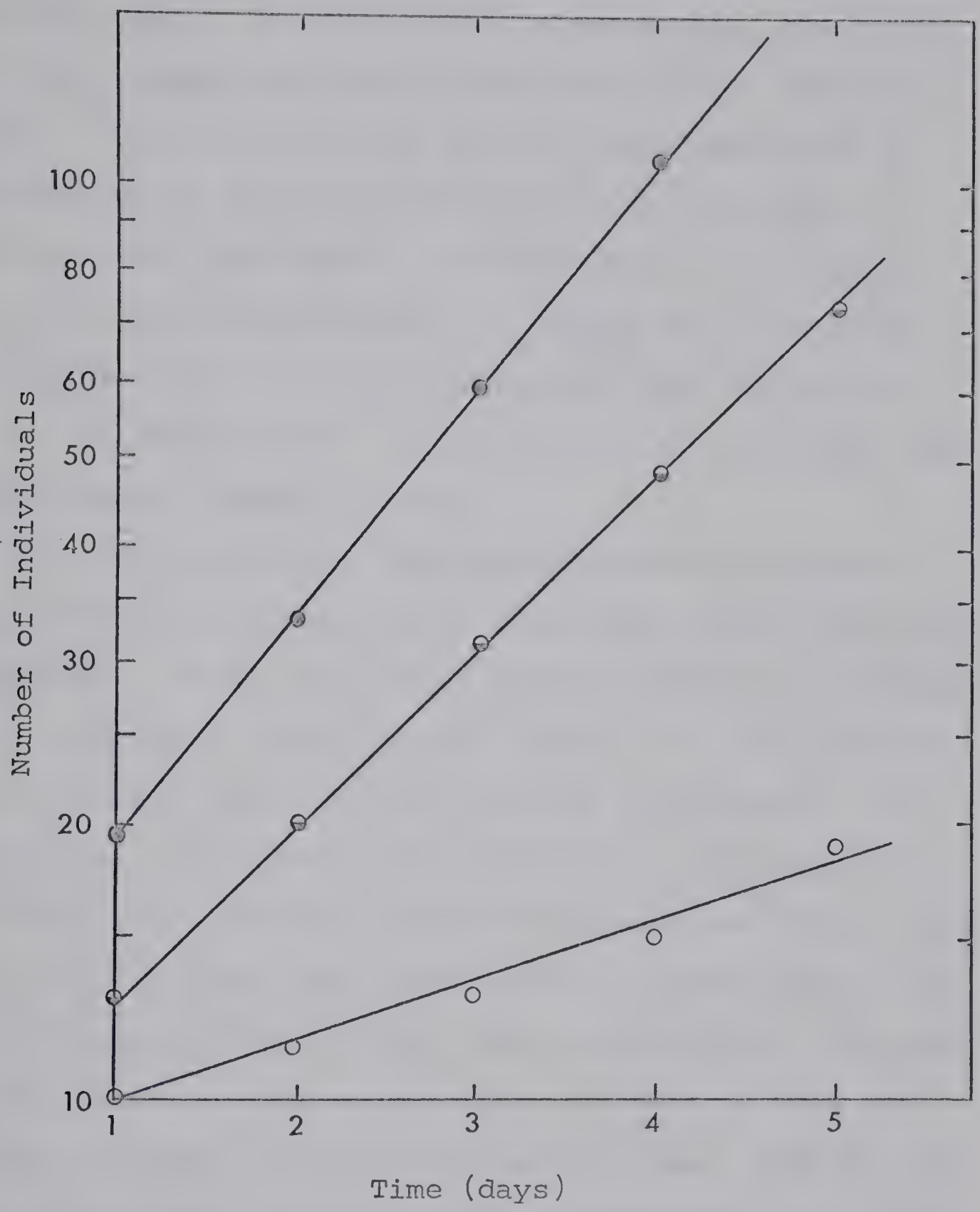

Figure 19. Growth rate curves for three species of Hydra. H. carnea data (O) from the present study. H. littoralis (●) and Chlorohydra viridissima (○) data from Loomis (1953).

increase in the number of individuals was observed for the first four days. As can be seen in the figure, the growth rate of H. carnea was slower than those of the other two species. Since very similar culture media were used in both studies and since the temperature in the study by Loomis was only one degree less than that in the present study, one might conclude that H. carnea has a naturally slower growth rate or that this species does not grow as well in the medium used. It may require an additional factor for optimum growth to occur.

The validity of the comparison between the present study and that by Loomis may be questioned on the basis of two factors. First, the method used by Loomis for studying growth rate was to place several hydra, each with numerous buds, in a dish and count the increase in hydranths. In this context, a hydranth is an individual with tentacles and mouth, thus including both mature hydra and buds. Thus, Loomis did not count only mature hydra, as was done in the present study, but he included buds in his count. The problem with counting both mature hydra and buds is that the two have different reproductive capabilities. Buds do not produce additional buds until after detachment, i.e., they do not begin budding until they are mature hydra. Also, Stiven (1962) has shown that newly detached individuals do not produce buds until 3-5 days after detachment.

The second problem with the study by Loomis is that he

only counted the increase in hydranths over a period of four days. Since the complete process of bud formation takes about two days and since the newly detached individuals do not begin to produce buds for 3-5 days, it seems that a period of four days is not sufficient time to adequately study the growth rate of hydra. This is substantiated by the present growth rate studies. The growth rate at the early part of each study was significantly different from that during the remainder of the study. This may be called an "induction period" and attributed to the time necessary for initial formation and maturation of buds.

As was shown in the previous chapter, when animals were cultured under blue, green, or red light, they showed a decreased sensitivity in terms of reaction time to the wavelength under which they were cultured, and they showed an increased sensitivity to other wavelengths when compared to the reaction times of animals that had been cultured in the dark. It appears that by continued exposure to light of a particular wavelength, the animals can be adapted to that wavelength so that they will no longer show a maximum sensitivity to it. Thus, by continuous stimulation with one wavelength, the animals show an increased reaction time to that wavelength. It also appears that light stimulation may enhance the general light sensitivity of the animals. For each of the three culture groups (blue, green, and red), the sensitivities to wavelengths other than that of the

culture light were higher than the sensitivities to those same wavelengths for animals cultured in the dark. It may be that animals cultured in the dark show a generally decreased sensitivity to all light because of a decrease in the functioning of the photoreceptive system due to a lack of stimulation by light.

The action spectrum of Hydra carnea cultured under white light can be compared to the results obtained by Singer, Rushforth and Burnett (1963) for H. pirardi. Figure 20 shows the reaction time vs. wavelength for these two species. The reaction times are the same at 550 nm but deviate sharply in either direction. The maximum sensitivities (shortest reaction times) of H. pirardi are at 425-500 nm, while those of H. carnea are at 600-675 nm. Since it has been shown that the composition of environmental light has an effect on spectral sensitivity, one might suggest that the two species were cultured under different light conditions. In fact, these two species were cultured under similar light conditions, consisting of cool white fluorescent tubes (Rushforth, personal communication), for these studies.

Other factors which may account for the differences in spectral sensitivity of the two species might involve inherent variation between the species. However, a review of numerous studies of other organisms fails to show much variation in the action spectra of animals in general.

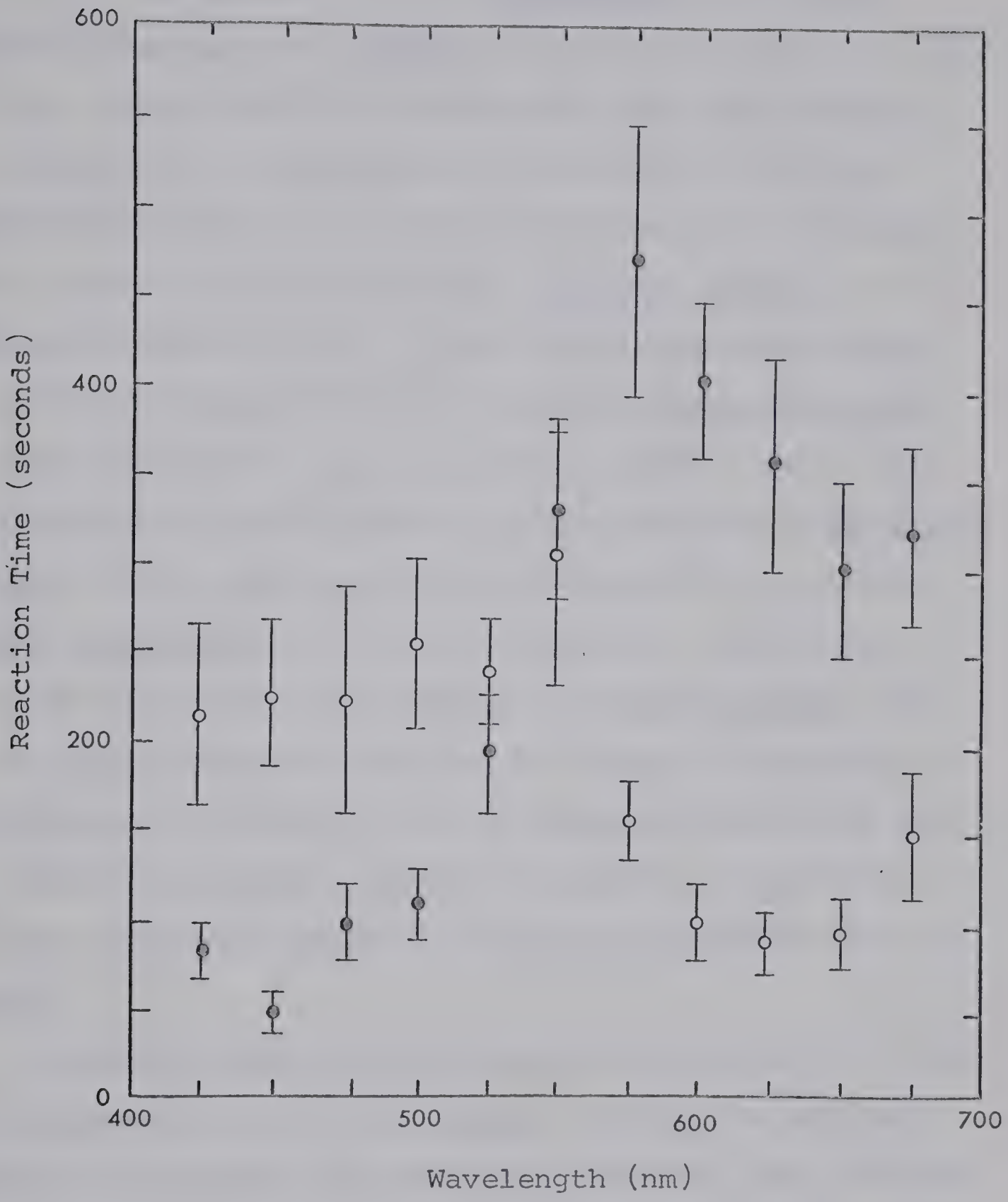

Figure 20. Reaction time *vs.* wavelength for two species of *Hydra*. Data for *H. carnea* (O) are from the present study; *H. pirardi* (O) from Singer *et al.* (1963).

Mast (1917) found the earthworm Lumbricus, the marine annelid Arenicola, and Euglena to be most sensitive to about 483 nm. Hecht (1920-1921) showed that the clam Mya was most sensitive to wavelengths in the region of 490 nm. Steven (1950) found the spectral sensitivity of the ammocoete larva of the brook lamprey, Lampetra planeri, to be greatest at about 525 nm. Steven (1955) reported 500-520 nm as the wavelengths to which the hag, Myxine glutinosa, was most sensitive. North and Pantin (1958) found a maximum sensitivity to 490-520 nm in the sea anemone Metridium senile. Yoshida and Millott (1960) found the sea urchin Diadema antillarum to be most sensitive to 455-460 nm. Thus, it appears that, in addition to Hydra pirardi, many other animals are most sensitive to light in the range of 425-525 nm. Therefore, it can be suggested that some factor other than inherent species variation is important in the very different pattern of sensitivity observed with H. carnea.

A possible factor contributing to the pattern of spectral sensitivity seen in H. carnea could be the northern latitude at which the specimens are located. Thus, variation in the natural solar radiation penetrating the atmosphere may account for the variation in spectral sensitivity. However, Myxine glutinosa and Lampetra planeri are both northern species. The greatest sensitivity for both these species was in the range 500-525 nm; it was at a greater

wavelength than that of other species reported but not at as great a wavelength as that found for Hydra carnea. Therefore, it is not possible at this time to determine the extent to which latitude may affect spectral sensitivity. Additional studies on other northern species might provide more information on this factor.

Another factor which may contribute to the variation in spectral sensitivity obtained for H. carnea is the fact that these animals came from artificially warmed waters. Although the animals used in this study were cultured in the laboratory at the same temperature as was used by Singer et al. (1963) in their study of the spectral sensitivity of H. pirardi, further studies to compare the sensitivities of warm and cold water species, including a study of H. carnea from waters which are not artificially warmed, are needed to determine the effect, if any, of the warm water on spectral sensitivity.

The question might arise as to the significance of the high sensitivity to red light found in this study for H. carnea. When evaluated on a quantum basis, this high sensitivity to red light is seen to be proportional to the higher number of quanta in red light at any given energy level in comparison to shorter wavelengths. Since growth rate is greater in red light, implying a higher metabolic rate, one would assume that a greater number of quanta are involved under red light than under blue or green light

when the total energy level is constant. It might be expected that if a similar study were conducted in which the number of quanta rather than the total energy of light were held constant, the growth rates and reaction times obtained would be the same at all wavelengths.

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APPENDIX I

Table XI

Growth rate data for hydra cultured under white light, showing the number of individuals every fifth day. The month shown is that in which the study was initiated.

	Study Number (October)		(November)		(December)		
Day	1	2	3	4	5	6	Total
1	1	1	1	1	1	1	6
5	1	1	2	1	3	2	10
10	2	3	4	2	5	4	20
15	6	6	9	5	5	8	39
20	7	9	15	7	16	14	68
25	9	14	17	10	18	17	85
30	18	20	24	19	22	23	126
35	30	42	39	34	45	30	220
40	46	61	59	55	60	45	326

Table XII

Growth rate data for hydra cultured under blue light.

	Study Number (October)		(November)		(December)		
Day	1	2	3	4	5	6	Total
1	1	1	1	1	1	1	6
5	1	1	1	1	1	1	6
10	2	2	4	3	3	3	17
15	5	5	6	6	7	7	36
20	9	7	10	9	9	9	53
25	13	13	13	14	14	14	81
30	17	18	16	19	17	17	104
35	23	24	21	23	21	25	137
40	28	32	29	30	31	32	182

Table XIII

Growth rate data for hydra cultured under green light.

	Study Number						
	(October)		(November)		(December)		
Day	1	2	3	4	5	6	Total
1	1	1	1	1	1	1	6
5	1	1	1	1	1	1	6
10	3	2	4	3	4	2	18
15	6	3	9	6	5	5	34
20	11	6	11	8	10	9	55
25	16	13	15	12	18	12	86
30	23	17	23	18	24	18	123
35	35	18	39	25	34	20	171
40	56	38	53	41	68	41	297

Table XIV

Growth rate data for hydra cultured under red light.

	Study Number						
	(October)		(November)		(December)		
Day	1	2	3	4	5	6	Total
1	1	1	1	1	1	1	6
5	1	1	2	1	1	1	7
10	3	3	5	6	3	3	23
15	4	13	10	12	8	8	55
20	18	24	16	37	24	15	134
25	28	42	32	46	52	28	228
30	49	68	57	72	62	40	348
35	68	83	78	87	94	70	480
40	104	148	111	143	150	112	768

APPENDIX II

Computer program for least-squares analysis.

```
C      THIS IS A GENERAL PROGRAM FOR LEAST SQUARE POLYNOMIAL FIT
C      FORTRAN IV MODIFICATION OF 001 LSQ1 FOR 360 OPERATION
C
C      DOUBLE PRECISION USED THROUGHOUT
C
C      LIST OF SYMBOLS USED FOR INPUT
C           N= NUMBER OF POINTS
C           M= NO. OF COEFFICIENTS OF POLY
C           N1= NO. OF ADDITIONAL POINTS AT WHICH EVALUATION OF FITTING
C               POLY. IS DESIRED.  MAX. N1=50
C           L= NO. OF DATA SETS TO BE FIT SIMULTANEOUSLY.  L=1-4
C           IEVAL= SIGNAL TO OUTPUT.  IF IEVAL>0, A TABLE OF THE INPUT
C                  DATA WITH POLY EVALUATIONS AND RESIDUALS IS PRINTED
C           X= INDEPENDENT VARIABLE
C           W= WEIGHTING FACTOR.  USE 1.0 FOR EQUAL WEIGHTING
C           Y= DEPENDENT VARIABLE
C           X1= EXTRA X AT WHICH POLY IS TO BE EVALUATED
C
      IMPLICIT REAL*8(A-H,O-Z)
      DIMENSION A(20,15), B(20,4), X(500), Y(500,4), W(500), SUM(4),
     X          RESID(500,4), STD(15,4), EVAL(500), X1(50)
C
    1 READ (5,1000) N, M, N1, L, IEVAL
      IF (N) 1,1,2
    2 DO 3 I = 1,N
    3 READ (5,2000) X(I), W(I), Y(I,1),Y(I,2),Y(I,3),Y(I,4)
      IF (N1) 5,5,4
    4 READ (5,2000)(X1(I), I = 1,N1)
    5 CALL LSQ1(X,Y,W,RESID,N,SUM,L,A,B,M)
      DEG = N - M - 1
      DO 6 I = 1,M
      DO 6 J = 1,L
    6 STD(I,J) =DSQRT(SUM(J) * A(1,1)/DEG)
      WRITE (6,3000) N, M, N1, L
      DO 7 I = 1,M
```

```
      7 WRITE (6,4000) (A(I,J), J = 1,M)
        DO 8 J = 1,L
        WRITE (6,5000) (B(I,J), STD(I,J), I = 1,M)
        WRITE (6,6000) SUM(J)
        IF (IEVAL) 11,11,9
      9 DO 10 I = 1,N
     10 EVAL(I) = RESID(I,J) + Y(I,J)
        WRITE (6,7000) (X(I),W(I),Y(I,J),EVAL(I),RESID(I,J),I = 1,N)
     11 IF (N1) 8,8,12
     12 DO 13 I = 1,N1
     13 EVAL(I) = PLLY(X1(I),M,B,J,20,4)
        WRITE (6,8000) (X1(I), EVAL(I), I = 1,N1)
      8 WRITE (6,9000)
        GO TO 1
   1000 FORMAT (12I6)
   2000 FORMAT (6D12.0)
   3000 FORMAT (48H1        LEAST SQUARE POLYNOMIAL FIT USING LSQ1  /
     X         3HON=I3,5H   M=I2,6H   N1=I2,5H   L=I2/13HOERROR MATRIX)
   4000 FORMAT (1HO1P8D14.5)
   5000 FORMAT (37HO          COEFFICIENT              ERROR/(1H 1P2D20.7))
   6000 FORMAT (36HOWEIGHTED SUM OF SQUARED DEVIATIONS=1PD13.5)
   7000 FORMAT (70HO          X              WEIGHT              Y          EVALUATI
     XON     RESIDUAL/(1H 1P5D14.5))
   8000 FORMAT (29HO     EXTRA X      EVALUATION/(1H 1P2D14.5))
   9000 FORMAT (54H - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -)
        END
```

```
      SUBROUTINE LSQ1(X,Y,W,RESID,N,SUM,L,A,B,M)
      IMPLICIT REAL*8(A-H,O-Z)
      DIMENSION X(500),Y(500,1),RESID(500,1),A(20,15),B(20,1),C(500,15),
     XSUM(1),W(500)
C
      COMMON C
C
      DO 1 I = 1,N
    1 C(I,1) = 1.0000000000
      DO 2 J = 2,M
      DO 2 I = 1,N
    2 C(I,J) = C(I,J - 1) * X(I)
      DO 3 I = 1,M
      DO 3 J = 1,M
      A(I,J) = 0.0000000000
      DO 3 K = 1,N
    3 A(I,J) = A(I,J) + C(K,I) * C(K,J) * W(K)
      DO 4 J = 1,L
      DO 4 I = 1,M
      B(I,J) = 0.0000000000
      DO 4 K = 1,N
    4 B(I,J) = B(I,J) + C(K,I) * Y(K,J) * W(K)
      CALL MATINV (A,M,B,L,DETERM)
      DO 6 J = 1,L
      SUM(J) = 0.0000000000
      DO 6 I = 1,N
      RESID(I,J) = POLY(X(I),M,B,J,20,1) - Y(I,J)
    6 SUM(J) = SUM(J) + RESID(I,J)**2*W(I)
      RETURN
      END
```

```
C
C       MATRIX INVERSION WITH ACCOMPANYING SOLUTION OF LINEAR EQUATIONS
C
        SUBROUTINE MATINV(A,N,B,M,DETERM)
C
        IMPLICIT REAL*8(A-H,O-Z)
        DIMENSION IPIVOT(20), A(20,20), B(20,1), INDEX(20,2), PIVOT(20)
        COMMON PIVOT,INDEX, IPIVOT
        EQUIVALENCE (IROW,JROW), (ICOLUM,JCOLUM), (AMAX,T,SWAP)
C
C       INITIALIZATION
C
        DETERM = 1.000000000
        DO 20 J = 1,N
     20 IPIVOT(J) = 0
        DO 550 I = 1,N
C
C       SEARCH FOR PIVOT ELEMENT
C
        AMAX = 0.000000000
        DO 105 J = 1,N
        IF(IPIVOT(J) - 1) 60,105,60
     60 DO 100 K = 1,N
        IF (IPIVOT(K) - 1) 80,100,740
     80 IF(DABS(AMAX) -DABS(A(J,K))) 85,100,100
     85 IROW = J
        ICOLUM = K
        AMAX = A(J,K)
    100 CONTINUE
    105 CONTINUE
        IPIVOT(ICOLUM) = IPIVOT(ICOLUM) + 1
C
C       INTERCHANGE ROWS TO PUT PIVOT ELEMENT ON DIAGONAL
C
        IF (IROW - ICOLUM) 140,260,140
    140 DETERM = -DETERM
```

```
      DO 200 L = 1,N
      SWAP = A(IROW,L)
      A(IROW,L) = A(ICOLUM,L)
  200 A(ICOLUM,L) = SWAP
      IF (M) 260,260,210
  210 DO 250 L = 1,M
      SWAP = B(IROW,L)
      B(IROW,L) = B(ICOLUM,L)
  250 B(ICOLUM,L) = SWAP
  260 INDEX(I,1) = IROW
      INDEX(I,2) = ICOLUM
      PIVOT(I) = A(ICOLUM,ICOLUM)
      DETERM = DETERM * PIVOT(I)
C
C     DIVIDE PIVOT ROW BY PIVOT ELEMENT
C
      A(ICOLUM,ICOLUM) = 1.000000000
      DO 350 L = 1,N
  350 A(ICOLUM,L) = A(ICOLUM,L)/PIVOT(I)
      IF (M) 380,380,360
  360 DO 370 L = 1,M
  370 B(ICOLUM,L) = B(ICOLUM,L)/PIVOT(I)
C
C     REDUCE NONPIVOT ROWS
C
  380 DO 550 L1 = 1,N
      IF(L1 - ICOLUM) 400,550,400
  400 T = A(L1,ICOLUM)
      A(L1,ICOLUM) = 0.0
      DO 450 L = 1,N
  450 A(L1,L) = A(L1,L) - A(ICOLUM,L) * T
      IF (M) 550,550,460
  460 DO 500 L = 1,M
  500 B(L1,L) = B(L1,L) - B(ICOLUM,L) * T
  550 CONTINUE
C
```

```
C       INTERCHANGE COLUMNS
C
        DO 710 I = 1,N
        L = N + 1 - I
        IF (INDEX(L,1) - INDEX(L,2)) 630,710,630
  630   JROW = INDEX(L,1)
        JCOLUM = INDEX(L,2)
        DO 705 K = 1,N
        SWAP = A(K,JROW)
        A(K,JROW) = A(K,JCOLUM)
        A(K,JCOLUM) = SWAP
  705   CONTINUE
  710   CONTINUE
  740   RETURN
        END
```



```
      REAL FUNCTION POLY(X,M,COEFF,J,MR,MC)
      IMPLICIT REAL*8(A-H,O-Z)
      DIMENSION COEFF(MR,MC)
      POLY = 0.0
      DO 1 N = 1,M
      MA = M - N + 1
    1 POLY = POLY * X + COEFF(MA,J)
      RETURN
      END
```

APPENDIX III

Computer program for determining the mean and standard deviation for a set of values with n = 25.

```
      DIMENSION TITLE(50),R(50)
      N=25
    3 READ(5,100) (TITLE(I),I=1,20)
      READ (5,200) (R(I), I=1,N)
      IF(R(1).EQ.0.0) GO TO 5
      SUM=0.0
      DO 1 I=1,N
    1 SUM=SUM+R(I)
      XM=SUM/N
      SD2=0.0
      DO 2 I=1,N
      DIFF2=(XM-R(I))**2
    2 SD2=SD2+DIFF2
      STD=SQRT(SD2/24)
      WRITE(6,300) (TITLE(I),I=1,20)
      WRITE(6,400) (R(I),I=1,N)
      WRITE(6,500) XM, STD
      GO TO 3
    5 STOP
  100 FORMAT(20A4)
  200 FORMAT(10F8.0)
  300 FORMAT(1H0,20A4/)
  400 FORMAT(15F8.2)
  500 FORMAT(8H0MEAN = ,F6.2,22H STANDARD DEVIATION = ,F6.2//)
      END
```

APPENDIX IV

Results of the "t" tests performed on the reaction time data.

Wavelength (nm)	Culture Groups* Being Compared	Calculated "t" value	Probability**
425	W - B	0.556	0.6
	W - G	5.109	0.001
	W - R	0.233	0.9
	B - G	6.087	0.001
	B - R	0.853	0.4
	G - R	5.194	0.001
450	W - B	6.716	0.001
	W - G	3.410	0.01
	W - R	1.633	0.2
	B - G	9.304	0.001
	B - R	6.676	0.001
	G - R	0.970	0.4
	WD - BD	0.925	0.4
	WD - GD	0.657	0.6
	WD - RD	0.077	p>0.9
	BD - GD	0.330	0.8
	BD - RD	1.002	0.4
	GD - RD	0.738	0.5
	W - WD	0.089	p>0.9
	B - BD	4.917	0.001
	G - GD	3.738	0.001
	R - RD	1.318	0.2

*In this appendix, W = White, B = Blue, G = Green, R = Red and D = Dark.

**The probability is less than the value given unless otherwise indicated and is the probability that the two means being compared are from the same population.

Wavelength (nm)	Culture Groups* Being Compared	Calculated "t" value	Probability**
475	W - B	5.667	0.001
	W - G	1.509	0.3
	W - R	2.171	0.05
	B - G	7.771	0.001
	B - R	3.900	0.001
	G - R	4.063	0.001
500	W - B	1.495	0.2
	W - G	2.268	0.05
	W - R	0.429	0.7
	B - G	0.695	0.5
	B - R	1.159	0.3
	G - R	1.955	0.1
525	W - B	3.477	0.01
	W - G	6.604	0.001
	W - R	1.570	0.2
	B - G	9.275	0.001
	B - R	4.228	0.001
	G - R	4.128	0.001
550	W - B	5.664	0.001
	W - G	4.586	0.001
	W - R	6.399	0.001
	B - G	3.627	0.001
	B - R	0.8197	0.5
	G - R	13.251	0.001
	WD - BD	0.773	0.5
	WD - GD	1.105	0.3
	WD - RD	0.551	0.6

*In this appendix, W = White, B = Blue, G = Green, R = Red and D = Dark.

**The probability is less than the value given unless otherwise indicated and is the probability that the two means being compared are from the same population.

Wavelength (nm)	Culture Groups* Being Compared	Calculated "t" value	Probability**
550	BD - GD	0.380	0.8
	BD - RD	0.262	0.8
	GD - RD	0.641	0.6
	W - WD	4.420	0.001
	B - BD	2.642	0.02
	G - GD	8.827	0.001
	R - RD	3.229	0.01
575	W - B	9.164	0.001
	W - G	9.268	0.001
	W - R	6.878	0.001
	B - G	0.141	0.9
	B - R	1.806	0.1
	G - R	1.699	0.1
600	W - B	19.565	0.001
	W - G	2.797	0.01
	W - R	14.040	0.001
	B - G	12.8496	0.001
	B - R	8.993	0.001
	G - R	6.352	0.001
625	W - B	10.042	0.001
	W - G	1.270	0.3
	W - R	13.274	0.001
	B - G	9.040	0.001
	B - R	3.618	0.001
	G - R	12.343	0.001
650	W - B	3.182	0.01
	W - G	2.557	0.02

*In this appendix, W = White, B = Blue, G = Green, R = Red and D = Dark.

**The probability is less than the value given unless otherwise indicated and is the probability that the two means being compared are from the same population.

Wavelength (nm)	Culture Groups* Being Compared	Calculated "t" value	Probability**
650	W - R	15.590	0.001
	B - G	0.262	0.8
	B - R	13.020	0.001
	G - R	12.385	0.001
	WD - BD	0.951	0.4
	WD - GD	0.772	0.5
	WD - RD	0.660	0.6
	BD - GD	0.119	p>0.9
	BD - RD	0.236	0.9
	GD - RD	0.109	p>0.9
	W - WD	6.410	0.001
	B - BD	2.897	0.01
	G - GD	2.743	0.01
	R - RD	9.486	0.001
675	W - B	0.087	p>0.9
	W - G	1.237	0.3
	W - R	4.153	0.001
	B - G	1.129	0.3
	B - R	3.318	0.01
	G - R	5.499	0.001

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